REMARKS

In the Official Action dated March 11, 2005, the Examiner did not enter the Amendment filed in response to the April 25, 2004 Office Action, alleging that the amendment raised new issues for appeal. Claims 1, 3, and 5-12 stand rejected under 35 U.S.C. §112 as allegedly indefinite. Claims 1, 3, and 5-12 stand rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention.

This response addresses each of the Examiner's objections and rejections. Accordingly, the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim 1 has been amended in this response for the purpose of expediting prosecution. Claims 7, 8, 11 and 12 have been cancelled without prejudice. No new matter has been added, and no amendments have been made in view of prior art. Entry of this amendment is respectfully requested.

The Examiner has rejected Claims 1, 3, and 5-12 under 35 U.S.C. §112 as allegedly indefinite. The Examiner has made the following rejections in this regard:

A. The Examiner rejected composition Claims 5 and 7 as allegedly substantial duplicates. For purposes of expediting prosecution, Applicants have cancelled claim 7 without prejudice. The cancellation of Claim 7 renders this rejection moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. The Examiner rejected composition Claims 6 and 8 as allegedly substantial duplicates. For purposes of expediting prosecution, Applicants have cancelled

Claim 8. The cancellation of Claim 8 renders this rejection moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

C. The Examiner rejected composition Claims 9 and 11 as allegedly substantial duplicates. For purposes of expediting prosecution, Applicants have cancelled Claim 11. The cancellation of Claim 11 renders this rejection moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

The Examiner rejected composition Claims 10 and 12 as allegedly substantial duplicates. For purposes of expediting prosecution, Applicants have cancelled Claim 12. The cancellation of Claim 12 renders this rejection moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

The Examiner has rejected Claims 1-3 and 5-12 under 35 U.S.C. §112, first paragraph, as allegedly lacking an enabling disclosure. The Examiner alleges that compounds having the ability to antagonize 5HT7 receptor sites are not necessarily enabling for the treatment of diseases listed in the claims.

Applicants respectfully submit that the clinical significance of compounds that have the ability to antagonize 5-HT7 receptor sites was known in the art at the time the application was filed. The connection between the 5-HT7 receptor and CNS disease such as depression and circadian rhythm disorders has been disclosed in a number of publications, including Lopez-Rodriguez, et. al., Bioorg. Med. Chem. Lett., 10 (2000) 1097-1100, a copy of which is enclosed for the Examiner's convenience.

In addition, all 5HT receptors belong to a large family of receptors that transduce signals through G proteins. These receptors are characterized pharmacologically by its high affinity for 5-HT. Physiological, clinical, and pharmacological studies have documented potential roles for the 5-HT1A receptor in neuroendocrine function and thermoregulation (Balcells-Olivero et al., 1998; Seletti et

al., 1995), vasoreactive headaches (Leone et al., 1998), sexual behavior (Maswood et al., 1998), food intake (Gilbert et al., 1988; Yamada et al., 1998), tooth-germ morphogenesis (Moisewitsch et al., 1998), memory (Edagawa et al., 1998), immune function (Iken et al., 1995), aggression (Miczek et al., 1998), depression (Blier et al., 1997; Shiah et al., 1998), and anxiety (Parks et al., 1998; Ramboz et al., 1998). See British Journal of Pharmacology 127, 1751–1764 (1999), a copy of which is submitted herein. Accordingly, this article provides credible evidence that the compounds of the present invention, which bind to 5-HT receptors and transduce signals through G proteins, can be useful for treating the specific diseases listed in the claims.

In addition, "Serotonin Receptors: Clinical Implications", Neuroscience and Behavioral Reviews, 14, 35-47 (1990), refers to the pharmacological effects associated with serotonin receptors including appetite suppression, thermoregulation, cardiovascular/hypotensive effects, sleep, psychosis, anxiety, depression, nausea, emesis, Alzheimer's disease, Parkinson's disease and Huntington's disease. A copy of this article is also enclosed for the Examiner's convenience.

Accordingly, the specification, as filed, discloses to one skilled in the art how to make and use the invention without undue experimentation.

Serotonin 7 partial agonists are useful for the treatment of depression. As used herein, the term "depression" includes depressive disorders, for example, single episodic or recurrent major depressive disorders, and dysthymic disorders, depressive neurosis, and neurotic depression; melancholic depression including anorexia, weight loss, insomnia and early morning waking, and psychomotor retardation; a typical depression (or reactive depression) including increased appetite, hypersomnia, psychomotor agitation or irritability, anxiety and phobias, seasonal affective disorder, or bipolar

disorders or manic depression, for example, bipolar I disorder, bipolar II disorder and cyclothymic disorder.

The Examiner requested test data for the compounds. In response, Applicants respectfully submit that there is no requirement, and it is not necessary to submit test data to satisfy 35 U.S.C. §112, first paragraph. Test data is not needed since, for the same reasons as stated above, the clinical significance of compounds that have the ability to antagonize 5HT7 receptor sites was known in the art at the time the application was filed. In addition Claim 4, which has been allowed in the instant case, lists a number of different compounds which exemplify a broad range of compounds that fall within the generic scope of Claim 1.

Furthermore, Applicants respectfully submit that there is sufficient direction and guidance to practice the claimed invention as disclosed throughout the specification as follows:

Page 7, paragraphs 94-97 and page 8, paragraphs 98-122, disclose, as an example, how to measure the affinities of the claimed compounds for serotonin 7 receptors.

Page 8, paragraph 123, discloses how measure the 5HT7 IC_{50} values for the claimed compounds.

Page 8, paragraphs 124-136 and page 9, lines 139-140, disclose how to evaluate the claimed compounds as 5HT7 receptors.

Page 8, paragraph 142, discloses how to determine the activity of the active compounds as antidepressants and related pharmacological properties.

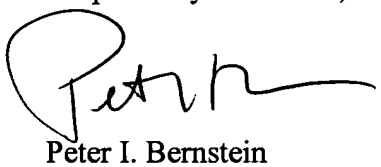
Applicants respectfully submit that based on the above disclosures in the present application, no undue experimentation is required to determine the utility and activity of the claimed compounds as antidepressants and related pharmacological

properties. A considerable amount of experimentation is permissible, if it is merely routine, or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. The present application provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Accordingly, the present application provides sufficient direction and guidance to the skilled artisan to practice the invention as claimed. In re Wands, 858 F.2d 731, 736-737, 8 U.S.P.Q. 1400, 1404 (Fed Cir. 1988). Necessary experimentation is not determinative of the question of enablement; only undue experimentation is fatal under the provisions of 35 U.S.C. §112, first paragraph. Id.

Therefore, it is respectfully submitted that the rejection under 35 U.S.C. §112, first paragraph, is overcome. Withdrawal of this rejection is respectfully requested.

Thus, in view of the foregoing amendments and remarks, the application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

First Pharmacophoric Hypothesis for 5-HT$_7$ Antagonism

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Abstract—In order to make the first contribution to the elucidation of essential structural features for 5-HT$_7$ antagonism, a set of thirty 5-HT$_7$ antagonists were selected from the literature. A pharmacophore model was built using Molecular Modeling studies with Catalyst program. The information contained in this model was validated with new synthesized compounds.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) was discovered over 50 years ago[1] and continues to generate interest as one of the most attractive targets for medicinal chemists. Molecular biological data have revealed the existence of fourteen serotonin receptor subtypes, which can be classified in seven families (5-HT$_{1-7}$).[2] The 5-HT$_7$ subtype is the most recent addition to the burgeoning family of 5-HT receptors.[3] Although the biological functions of the 5-HT$_7$ receptor are poorly understood, preliminary evidences suggest that it may be involved in depression,[4] control of circadian rhythms,[5] and relaxation in a variety of vascular smooth muscles.[6] Nevertheless, the therapeutic utility of 5-HT$_7$ receptor ligands awaits the development of selective agonists and antagonists. During our work only two selective 5-HT$_7$ receptor antagonists (SB-258719[7] and DR4004[8]) were discovered, both from a high-throughput screening of compound libraries. In the meantime of editorial revision, Lovell et al.[9] have reported SB-269970 as a new selective antagonist structurally related to SB-258719. Information on the structural requirements of 5-HT$_7$ ligands is still unknown and its determination is the major aim for developing specific compounds. In a rational drug design, identification of the pharmacophore is one of the most important steps, especially when the structure and properties of the bioreceptor remain unknown. Therefore, our aim in this communication is to report the essential structural features for 5-HT$_7$ antagonism. The validation of the pharmacophore using data of new synthesized compounds suggests consistencies in structural requirements.

Pharmacophore Generation

The study was performed using the software package Catalyst[10] installed on a Silicon Graphics O2 workstation. A set of thirty 5-HT$_7$ antagonists[7,8,11–17] structurally different from a chemical feature standpoint was selected from the reported data as the target training set for Catalyst analysis (Tables 1–4). In cases where the chirality of a stereogenic center was not specified, Catalyst generated and considered alternative stereoisomers. All structures were built de novo using 2D/3-D editor sketcher in Catalyst. Conformational models were calculated using a 15 Kcal energy cutoff (minimization convergence criteria during conformational analysis: energy convergence = 0.1 Kcal/mol/Å, gradient convergence = 0.01 Kcal/mol/Å). The number of conformers generated for each substrate was limited to a maximum of 250. All molecules with their associated conformations were regrouped including the biological data (pK_i). Hypothesis generation was performed and twelve hypotheses were obtained using low energy conformers of the molecules in the training set. After assessing all generated hypotheses, the most plausible one was considered the best. The goodness of the structure–activity correlation was estimated by means of r^2.

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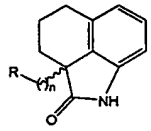
Table 1. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore

Number	Compound	pK_i (5-HT$_7$)	References
1	Metergoline	8.2	12, 13, 14
2	Mesulergine	8.1	12
3	2-Br-LSD	8.0	11a
4	Methysergide	7.9	12
5	Clozapine	7.9	12
6	(*S*)-Methiothepin	9.0	12
7	Cyproheptadine	7.3	12
8	Mianserin	7.2	15
9	(+)-Butaclamol	7.2[a]	11a, 15
10	Ritanserin	7.8	12
11	Spiperone	7.7	15, 16

[a]This value represents the mean of different pK_i values reported in refs 11a and 15.

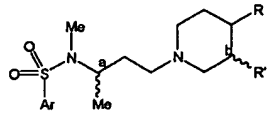
Table 2. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore



Number	*n*	R	pK_i (5-HT$_7$)[a]
12	2	4-phenylpiperazin-1-yl	7.0
13	3	4-phenylpiperazin-1-yl	8.3
14	4	4-phenylpiperazin-1-yl	8.5
15	4	4-(2-methoxyphenyl)piperazin-1-yl	8.3
16	4	4-(2-cyanophenyl)piperazin-1-yl	8.4
17	4	4-(2-pyridyl)piperazin-1-yl	8.7
18 (DR4004)	4	4-phenyl-1,2,3,6-tetrahydropyridyl	8.7
19	4	4-cyclohexylpiperazin-1-yl	5

[a]Values reported in ref 8.

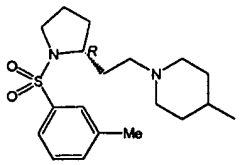
Table 3. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore



Number	Ar	R	R′	Stereochemistry a	Stereochemistry b	pK_i (5-HT$_7$)[a]
20	1-naphthyl	H	Me	*R,S*	*R,S*	7.2
21	1-naphthyl	H	Me	*R*	*R*	6.9
22	1-naphthyl	H	Me	*R*	*S*	6.2
23	1-naphthyl	H	Me	*S*	*R*	5.8
24	1-naphthyl	H	Me	*S*	*S*	5
25 (SB-258719)	3-methylphenyl	Me	H	*R*	—	7.5
26	1-naphthyl	Me	H	*R*	—	7.5
27	3,4-dichlorophenyl	Me	H	*R*	—	7.5
28	3,4-dibromophenyl	Me	H	*R*	—	7.7
29	4,5-dibromo-2-thienyl	Me	H	*R*	—	7.8

[a]Values reported in ref 7.

Table 4. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore

Number	Compound	pK_i (5-HT$_7$)[a]
30		8.5

[a]Value reported in ref 17.

Results

According to the hypothesis generated by catalyst, the minimal structural requirements for 5-HT$_7$ antagonism consist of an aromatic ring, a basic nitrogen atom (positive ionizable center), a H-bonding acceptor group and a hydrophobic region at 4.9–5.9 Å apart from the basic center (Fig. 1). For all the molecules in the training set, reasonable low-energy conformers that align on the hypothesis were found. The overall ability of this hypothesis to estimate properly the affinities of all molecules within the training set is shown by the good r^2 value between predicted and estimated affinities ($r^2 = 0.921$). This pharmacophoric assumption was then validated using new naphtholactam and naphthosultam derivatives (Fig. 2). Affinity data (Table 5) suggest consistencies in required structural features.

Compounds **31–45**[18] were obtained by treatment of intermediates **46** with the corresponding piperazines and piperidines **47** in the presence of triethylamine and ace-

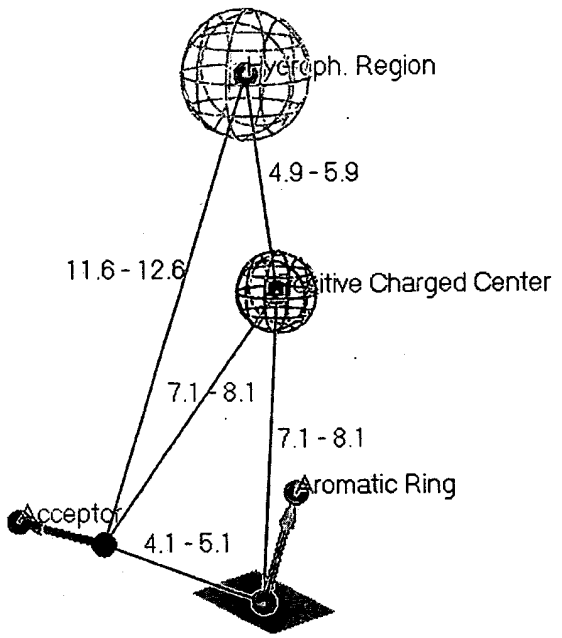


Figure 1. Proposed pharmacophore for 5-HT$_7$ antagonism.

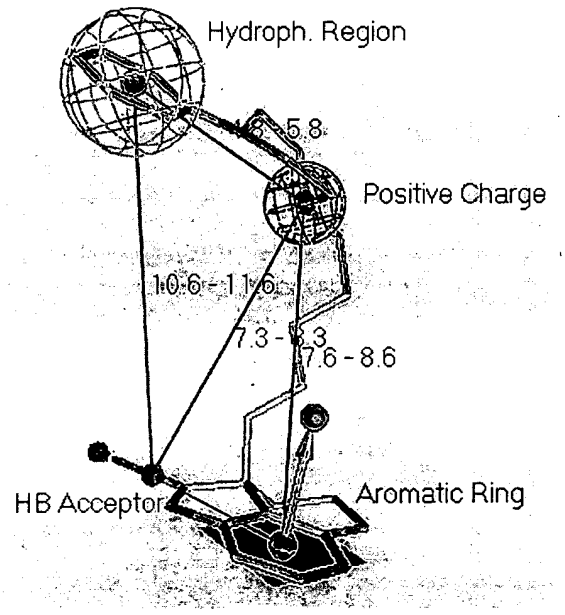
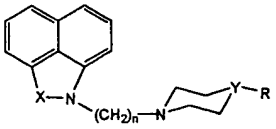


Figure 2. Compound **42** mapped on the hypothesis generated for 5-HT_7 receptor antagonists.

Table 5. Binding affinity of synthesized compounds at 5-HT_7 receptors.[a]



Compd	X	*n*	Y	R	pK_i[a]
31	CO	4	CH	H	< 5
32	CO	4	CH	Me	< 6
33	CO	4	N	Me	< 5
34	SO_2	4	CH	H	< 5
35	SO_2	4	CH	Me	< 5
36	SO_2	4	N	Me	< 5
37	SO_2	5	CH	Me	< 5
38	CO	4	CH	isopropyl	6.7
39	CO	4	N	*o*-methoxyphenyl	7.2
40	SO_2	4	N	*o*-methoxyphenyl	6.7
41	CO	5	N	*o*-methoxyphenyl	7.0
42	CO	5	N	phenyl	7.1
43	SO_2	5	N	*o*-methoxyphenyl	6.6
44	SO_2	5	N	phenyl	6.7
45	SO_2	6	N	*o*-methoxyphenyl	6.7

[a]pK_i = −log K_i. K_i (nM) values are means of two to four assays, performed in triplicate. Inhibition curves were analyzed by a computer-assisted-curve-fitting program (Prism GraphPad) and K_i values were determined from the Cheng–Prusoff equation.

tonitrile (Scheme 1). The reaction of compounds **48** with the appropriate dibromoderivative rendered key intermediates **46**. Respective hydrochloride salts of the synthesized compounds were prepared as samples for biological assays.

Target compounds were assessed for in vitro affinity at the 5-HT_7 receptor by radioligand binding assays, using [^{3}H]-5-CT in rat hypothalamus membranes[19] (Table 5). All the compounds that fit our pharmacophore model (**38–45**) show 5-HT_7 affinity (pK_i >6.5), whereas all inactive derivatives (**31–37**, pK_i < 6.5) lack the hydrophobic moiety situated at a distance of 4.9–5.9 Å from the nitrogen atom, required for 5-HT_7 binding. These results support our pharmacophoric hypothesis. In particular, compound **42** (Fig. 2) has been selected as a new lead compound for the search for 5-HT_7 receptor ligands.

Conclusions

The pharmacophore model for 5-HT_7 antagonism described herein represents the first contribution to the rational design of agents acting at this recently identified serotonin receptor. The postulated hypothesis was validated with a series of new naphtholactam and naphthosultam derivatives that exhibit affinity for the 5-HT_7 receptor. This study offers structural insight to aid the development of novel 5-HT_7 ligands, which are essential for the knowledge of the (patho)physiological role of this serotonin receptor subtype.

Acknowledgements

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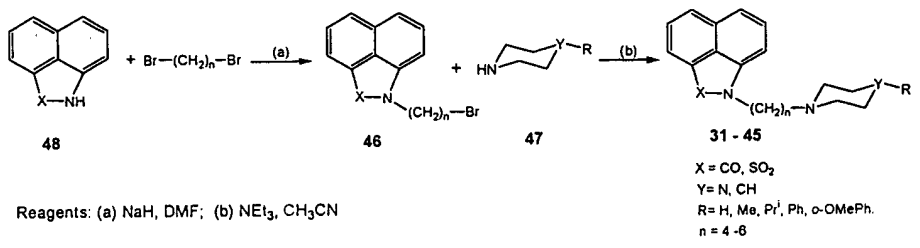

Scheme 1.

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18. New compounds were characterized by IR, ^{1}H, and ^{13}C NMR spectroscopy and gave satisfactory combustion analysis (C, H, N). Spectral data of selected compound **42**: IR ($CHCl_3$, cm^{-1}) 1705 (CON), 1600, 1560, 1496, 1456 (Ar); ^{1}H NMR ($CDCl_3$) δ 1.43 (qt, $J=7.2$ Hz, 2H, CH_2), 1.58 (qt, $J=7.5$ Hz, 2H, CH_2), 1.82 (qt, $J=7.5$ Hz, 2H, CH_2), 2.35 (t, $J=7.5$ Hz, 2H, CH_2N–pip), 2.55 (t, $J=5.1$ Hz, 4H, $2CH_2$-pip), 3.15 (t, $J=5.1$ Hz, 4H, $2CH_2$-pip), 3.92 (t, $J=7.5$ Hz, 2H, CH_2–NCO), 6.83 (t, $J=7.2$ Hz, 1H, H_4-phenyl), 6.90 (d, $J=7.8$ Hz, 3H, H_2-, H_6-phenyl, H_8), 7.24 (t, $J=6.9$ Hz, 2H, H_3-, H_5-phenyl), 7.45 (t, $J=8.4$ Hz, 1H, H_7), 7.52 (d, $J=8.4$ Hz, 1H, H_6), 7.69 (t, $J=8.1$ Hz, 1H, H_4), 7.99 (d, $J=8.1$ Hz, 1H, H_5), 8.04 (d, $J=7.2$ Hz, 1H, H_3); ^{13}C NMR ($CDCl_3$) δ 24.8, 26.5, 28.6 (CH_2–CH_2–CH_2), 40.1 ($\underline{C}H_2$–NCO), 49.0 ($2CH_2$-pip), 53.2 ($2CH_2$-pip), 58.4 (CH_2N-pip), 104.8 (C_8), 115.9 (C_2-, C_6-phenyl), 119.5 (C_4-phenyl), 120.1 (C_6), 124.1 (C_3), 125.1 (C_{8b}), 126.7 (C_{2a}), 128.4, 129.0 (C_4, C_7, C_3-, C_5-phenyl), 128.6 (C_{5a}), 130.6 (C_5), 139.5 (C_{8a}), 151.2 (C_1-phenyl), 168.0 (CO); mp 211–213 °C (CH_2Cl_2:hexane)..
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REVIEW ARTICLE

The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways

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The 5-hydroxytryptamine 5-HT_{1A} receptor was one of the first G protein coupled receptors whose cDNA and gene were isolated by molecular cloning methods. Transfection of the cDNA of this receptor into cells previously bearing no 5-HT receptors has resulted in the acquisition of large amounts of information regarding potential signal transduction pathways linked to the receptor, correlations of receptor structure to its various functions, and pharmacological properties of the receptor. Transfection studies with the 5-HT_{1A} receptor have generated critical new information that might otherwise have been elusive. This information notably includes the discovery of unsuspected novel signalling linkages, the elucidation of the mechanisms of receptor desensitization, the refinement of models of the receptor pharmacophore, and the development of silent receptor antagonists, among others. The current review summarizes the most important studies of the recombinant 5-HT_{1A} receptor in the decade since the identificiation of its cDNA.

Keywords: 5-Hydroxytryptamine; transfection; G protein; adenylyl cyclase; phospholipase; calcium; efficacy; potency

Abbreviations: cyclic AMP, 3',5'-cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane regulator; DAG, diacylglycerol; Erk, extracellular signal-regulated kinase; G protein, guanine nucleotide regulatory binding protein; G_i, G protein that inhibits adenylyl cyclase; GIRK, G protein-gated inwardly rectified K^+ channel; G_o, G protein that serves functions other than to regulate adenylyl cyclase; G_q, G protein that activates phospholipase C; Grb2, protein that serves as a molecular adapter; GRK, G protein-coupled receptor kinase; GTP, guanosine triphosphate; GTPγS, nonhydrolysable GTP analogue; G_S, G protein that stimulates adenylyl cyclase; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)-tetralin; 5-HT, 5-hydroxytryptamine; serotonin; i2 loop, putative second intracellular loop of the 5-HT_{1A} receptor protein; i3 loop, putative third intracellular loop of the 5-HT_{1A} receptor protein; IκBα, inhibitor of NF-κB; IP_3, inositol triphosphate; Mek, mitogen and extracellular signal regulated kinase, which phosphorylates and activates Erk; NF-κB, nuclear factor-κB; PC-PLC, phosphatidylcholine-specific phospholipase C; PI-PLC, phosphatidylinositol-specific phospholipase C; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; Raf; a kinase that is activated by Ras, and that phosphorylates and activates MEK; Ras, a monomeric low molecular weight G protein that activates Raf; RGS proteins, regulators of G protein signalling; ROI, reactive oxygen intermediates; R-SAT, receptor selection and amplification technology; Shc, a protein that serves as a docking platform; Src, non-receptor tyrosine kinase; WAY-100635, N-[2-[4-(2-methoxyphenyl)1-piperaziny-l]ethyl)-n-(2-pyridiynl)cyclohexanecarboxamide trihydrochloride

Introduction

Serotonin (5-HT, 5-hydroxytryptamine) was discovered in 1948 by Rapport *et al.* as a potent vasotonic factor. For several decades, many of the effects of 5-HT were attributed to two major subtypes of 5-HT receptors (Gaddum & Picarelli, 1957). With the development in the 1980s of specific ligands for the various subtypes of 5-HT receptors came the realization that there must be more than two subtypes of 5-HT receptor. Molecular cloning studies over the last decade have confirmed the existence of at least 14 subtypes of 5-HT receptors, each encoded by a distinct gene. Most of those receptors belong to a large family of receptors that transduce signals through G proteins.

One of the best-characterized 5-HT receptors is the 5-HT_{1A} receptor (Pedigo *et al.*, 1981). This receptor is characterized pharmacologically (like all 5-HT_1 receptors) by its high affinity for 5-HT. It also has a uniquely high affinity for 8-OH-DPAT, and for second generation, arylpiperazine anxiolytic agents such as buspirone, gepirone and ipsapirone. Physiological, clinical, and pharmacological studies have documented potential roles for the 5-HT_{1A} receptor in neuroendocrine function and thermoregulation (Balcells-Olivero *et al.*, 1998; Seletti *et al.*, 1995), vasoreactive headaches (Leone *et al.*, 1998), sexual behaviour (Maswood *et al.*, 1998), food intake (Gilbert *et al.*, 1988; Yamada *et al.*, 1998), tooth-germ morphogenesis (Moisewitsch *et al.*, 1998), memory (Edagawa *et al.*, 1998), immune function (Iken *et al.*, 1995), aggression (Miczek *et al.*, 1998), depression (Blier *et al.*, 1997; Shiah *et al.*, 1998) , and anxiety (Parks *et al.*, 1998; Ramboz *et al.*, 1998).

The 5-HT_{1A} receptor was one of the first G protein-coupled receptors for which the cDNA and gene were cloned (Albert *et al.*, 1990; Chanda *et al.*, 1993; Fargin *et al.*, 1989; Fujiwara *et al.*, 1990; Kobilka *et al.*, 1987; Stam *et al.*, 1992). This gene is intronless, and its message is expressed mainly in the brain, spleen, and neonatal kidney (Fargin *et al.*, 1989; Kobilka *et al.*, 1987). A number of interesting observations have been derived

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from the primary nucleic and amino acid sequences of the 5-HT$_{1A}$ receptor. The rat and human 5-HT$_{1A}$ receptor nucleic acid sequences are 88% homologous with each other (Albert *et al.*, 1990; Fargin *et al.*, 1989; Fujiwara *et al.*, 1990; Kobilka *et al.*, 1987; Stam *et al.*, 1992), whereas those sequences possess significantly less homology with other members of the family of G protein-coupled 5-HT receptors such as the 5-HT$_{2A}$ (19%) and 5-HT$_{2C}$ (18%) receptors and the 5-HT$_{1D}$ receptors (43%) (Fujiwara *et al.*, 1993). The potential importance of the 5-HT$_{1A}$ receptor has been underscored by the recent cloning of putative homologues in non-mammalian species such as *Xenopus laevis* (Maracci *et al.*, 1997) and *Fugu rubripes* (Yamaguchi & Brenner, 1997).

The encoded human protein, composed of 422 amino acids is characterized by a core molecular weight of ≈46,000, and an isoelectric point of 8.8. Hydropathicity analysis reveals that the 5-HT$_{1A}$ receptor contains seven hydrophobic stretches that could possibly form membrane-spanning α-helices. By analogy with the β_2-adrenoceptor, and because of the presence of three consensus sequences for *N*-linked glycosylation on the amino terminus, the receptor is probably oriented in the plasma membrane with the amino terminus facing the extracellular domain. Hydrophilic sequences that form three intracellular and three extracellular loops connect the seven hydrophobic transmembrane regions. In the putative second extracellular domain the 5-HT$_{1A}$ receptor possesses a cysteine residue (Cys^{186}) which may form a disulfide bond with Cys^{109}, which is located at the limit between the first extracellular loop and the third transmembrane domain (Figure 1). By analogy with what has been shown for the β_2-adrenoceptor (Dohlman *et al.*, 1990; Fraser *et al*, 1989), this disulfide bond may stabilize the receptor conformation and explain in part, why reducing agents affect 5-HT$_{1A}$ receptor binding properties (Emerit *et al.*, 1991; Gozlan *et al.*, 1988). It should be noted that the original sequence of the human receptor (Kobilka *et al.*, 1987) was modified slightly by Chanda *et al.* (1993), because the initial publication of Kobilka *et al.* (1987) appears to have contained a sequencing error near the junction of the second intracellular loop and the fourth transmembrane domain.

The 5-HT$_{1A}$ receptor is quite interesting in that it has been implicated in numerous signalling pathways in physiologically relevant model systems. In nearly every case, the signals have been shown to be exquisitely sensitive to pertussis toxin, exclusively implicating $G_{i/o}$ protein in signals initiated by the 5-HT$_{1A}$ receptor in physiological settings. In neurons, the major signals emanating from the 5-HT$_{1A}$ receptor are inhibition of adenylyl cyclase (De Vivo & Maayani, 1986; Weiss *et al.*, 1986), and opening of K^+ channels (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Zgombick *et al.*, 1989). The 5-HT$_{1A}$ receptor has also been demonstrated to inhibit a Ca^{2+} current (Pennington & Kelly, 1990), to stimulate adenylyl cyclase (Markstein *et al.*, 1986; Shenker *et al.*, 1985), and to inhibit phospholipase C activation (Claustre *et al.*, 1988) in various neuronal preparations. In non-neuronal rat ventral prostate cells, endogenous 5-HT$_{1A}$ receptors inhibit adenylyl cyclase and stimulate nitric oxide synthase (Carmena *et al.*, 1998).

The realization that the 5-HT$_{1A}$ receptor couples to multiple signalling pathways in cells and tissues in which it is normally expressed reflects a relatively new understanding of the potential promiscuity of receptor-G protein signalling pathways. Nevertheless, it has long been suspected that single receptor subtypes might be linked to various second messengers in a single cell system (Limbird, 1988). No matter how carefully constructed the experiments, however, there is always the consideration in complex tissues and organs that the effects may be mediated by more than one receptor subtype, which are pharmacologically similar but functionally distinct. These considerations can be eliminated by inserting a receptor through transfection methods into a cellular model which previously lacked that or related receptors. Transfected cells have the advantage of expressing single well-defined receptor subtypes. Non-transfected or dummy-transfected cells serve as excellent controls. The purpose of this review is to summarize information that has been obtained by transfection of the recombinant 5-HT$_{1A}$ receptor into various cell models. We will focus first upon issues relevant to signal transduction and then upon other functions of the 5-HT$_{1A}$ receptor.

Signalling linkages

Coupling of the 5-HT$_{1A}$ receptor to many of the signalling pathways described in tissues has been recapitulated in various transfected cell lines, with two notable exceptions. First, positive coupling of the 5-HT$_{1A}$ receptor to adenylyl cyclase has not been documented in any transfected cell line to date. Various manoeuvres in CHO cells, including prolonged treatment with pertussis toxin and/or co-stimulation with $G_{s\alpha}$-coupled receptors failed to reveal a positive regulation of adenylyl cyclase or cellular cyclic AMP levels. Further, no direct coupling between the 5-HT$_{1A}$ receptor and $G_{s\alpha}$ has been detected with G protein photoaffinity labelling, cholera toxin catalyzed ADP-ribosylation reactions, or co-immunoprecipitation experiments (Raymond *et al.*, 1993b; and unpublished observations). Second, coupling to nitric oxide has not been described in transfected, cultured cells, but the discovery of this pathway in rat ventral prostate was only recently described (Carmena *et al.*, 1998).

Inhibition of adenylyl cyclase

The most consistent coupling of the recombinant 5-HT$_{1A}$ receptor is to the inhibition of adenylyl cyclase through pertussis toxin-sensitive G proteins. The coupling to the inhibition of adenylyl cyclase appears to be universally expressed (Banerjee *et al.*, 1993; Fargin *et al.*, 1989; Liu & Albert, 1991; Varrault *et al.*, 1992b), and is extremely efficient in that the efficacy of coupling is maximal at low physiologically relevant levels of receptor expression. The efficiency of coupling is probably due to abundant receptor reserve. Langlois *et al.* (1996) demonstrated that 5-HT$_{1A}$ receptors transfected into polarized epithelial LLC-PK$_1$ cells were expressed on both basolateral and apical membranes. Receptors on both surfaces were able to inhibit cyclic AMP accumulation. Thus, the recombinant 5-HT$_{1A}$ receptor has been shown to consistently inhibit adenylyl cyclase in multiple cells, and also on two key membrane domains in a polarized cell line.

Despite its obvious importance, the mechanism of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor remains largely unexplored. The family of adenylyl cyclases consists of at least nine distinct members, each possessing a diverse repertoire of regulatory inputs (Hurley, 1999; Mons *et al.*, 1998; Taussig & Zimmerman, 1998). Because the 5-HT$_{1A}$ receptor can activate a multitude of signalling pathways (described below), it could regulate adenylyl cyclase in a number of ways depending both upon the specific signals generated by receptor occupancy, and upon the types and amounts of adenylyl cyclases expressed in the cells or tissues of interest. For example, $G_{i\alpha}$ subunits bind to and inhibit types 5 and 6 adenylyl cyclase. This would seem to be a rather

straightforward means of regulation of adenylyl cyclase by the 5-HT$_{1A}$ receptor since this receptor almost universally couples to $G_{i\alpha}$ subunits. However, $G_{\beta\gamma}$ subunits can conditionally stimulate type 2 adenylyl cyclase when activated $G_{s\alpha}$ is present. Elevations of intracellular Ca^{2+} also can inhibit types 5 and 6 adenylyl cyclase. In contrast, activation of Ca^{2+}/calmodulin can stimulate types 1, 3, and 8 of adenylyl cyclase. Protein kinase C can stimulate types 2 and 7 adenylyl cyclase, whereas Ca^{2+}/calmodulin-dependent protein kinase II can inhibit type 3 adenylyl cyclase (Hurley, 1999; Mons *et al.*, 1998; Taussig & Zimmerman, 1998). Moreover, the 5-HT$_{1A}$ receptor could regulate cellular levels of cyclic AMP downstream of adenylyl cyclase by inhibiting its destruction by phosphodiesterases or other mechanism (Wang *et al.*, 1999). Thus, what on the surface appears to be a rather straightforward regulation of adenylyl cyclase by the 5-HT$_{1A}$ receptor may in fact be a complex summation of a number of distinct regulatory signals. Transfected cell systems hold promise for exploring this issue

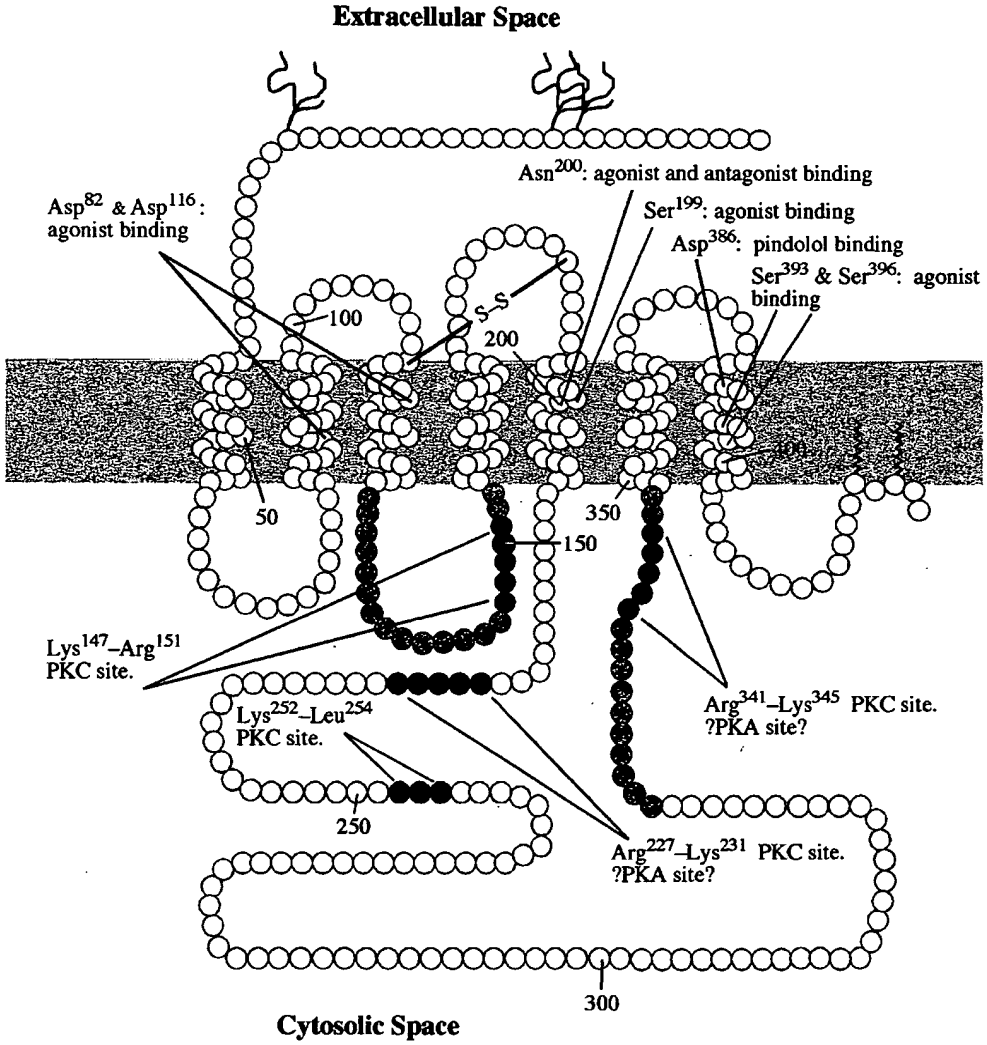

Figure 1 Two-dimensional topographical representation of the human 5-HT$_{1A}$ receptor. Each circle represents an amino acid. Numbering of the amino acids commences from the initiator methionine, and every 50 amino acids are marked for convenience. The amino terminus faces the extracellular space, and the carboxyl terminus faces the cytoplasm. Seven transmembrane stretches, each composed of $\approx$20–26 hydrophobic amino acids, are connected by three extracellular loops (termed e1, e2, and e3) and three intracellular loops (termed i1, i2, and i3). There are three potential sites of *N*-linked glycosylation on the amino terminus (depicted as branching trees). A disulfide bond between Cys^{109} and Cys^{187} putatively links the e1 and e2 loops. Transmembrane (TM) domains contain residues important for ligand binding. Asp^{82} (in TM2) and/or Asp^{116} (in TM3) are important for binding of 5-HT, perhaps by serving as a counterion for the amine group. Ser199 (in TM5) also is important for binding of 5-HT. Asn^{200} (in TM5) is important both for agonist and antagonist binding. Asp^{386} (in TM7) is critical for selective binding to β-adrenoceptor blocker like pindolol. Ser^{393} and Ser^{396} (also in TM7) are important for agonist binding. The intracellular domains contain sites that are important for contacting G proteins particularly the entire i2 loop and the carboxy terminal end of the i3 loop (gray circles). Sites putatively involved in phosphorylation by PKC and PKC are depicted as black circles. Specific PKC phosphorylation sites are as follows: Lys^{147}-Arg-Thr-Pro-Arg^{151} (i2); Arg^{227}-Lys-Thr-Val-Lys^{231} (i3); Lys^{252}-Ser-Leu^{254} (i3); and Arg^{341}-Lys-Thr-Val-Lys^{345} (i3). There are two putative PKA sites: Arg^{227}-Lys-Thr^{229} and Arg^{341}-Lys-Thr^{343}. Underlined residues are putative phosphate acceptor sites. The carboxy terminal tail is putatively anchored to the interior face of the plasma membrane by one or two palmitoyl anchors bound to Cys^{418} and/or Cys^{420}.

because they offer the ability to alter the amount and types of adenylyl cyclase present within the host cell.

Coupling to phospholipases

Activation of phosphatidylinositol-specific phospholipase C (PLC) results in the generation of two key second messengers. The first is inositol trisphosphate (IP_3), which regulates intracellular Ca^{2+} release (Berridge *et al.*, 1998). The second is diacylglycerol (DAG), which binds to and activates protein kinase C (PKC). PLC can be activated by receptors that couple to both pertussis toxin-sensitive and -insensitive G proteins. Activation of PLC by G_q-coupled 5-HT$_2$ receptors appears to be almost universal. Although 5-HT$_{1A}$ receptors can clearly activate PLC, this effect is highly host cell-specific. Fargin *et al.* (1989) first demonstrated that the 5-HT$_{1A}$ receptor could activate PI-PLC in HeLa cells. This coupling was shown to be as effective as that induced by endogenous histamine H_1-like receptors (Raymond *et al.*, 1989). However, this coupling was not as efficient as coupling to the inhibition of adenylyl cyclase in that the EC_{50} of PLC stimulation occurred at significantly higher ligand concentrations, and because virtually no receptor reserve was apparent. This coupling may be physiologically relevant in that endogenous 5-HT$_{1A}$ receptors in human Jurkat (T cell-like) cells activates PLC (Aune *et al.*, 1993). Liu & Albert (1991) were the first to demonstrate that activation of PI-PLC by the 5-HT$_{1A}$ receptor is cell-specific. They showed that the 5-HT$_{1A}$ receptor expressed in Ltk$^-$ fibroblasts activates PI-PLC because 5-HT increased phosphoinositide hydrolysis and levels of intracellular Ca^{2+}. In BALB/c-3T3 cells and in Ltk$^-$ fibroblasts, 5-HT$_{1A}$ receptor-mediated increases in intracellular Ca^{2+} derive from the release of intracellular Ca^{2+}, rather than from influx of extracellular Ca^{2+} (Abdel-Baset, 1992). In contrast, when the 5-HT$_{1A}$ receptor was expressed in GH_4C_1 pituitary cells, no evidence of PI-PLC activity was detected (Liu & Albert, 1991). Interestingly when the human 5-HT$_{1A}$ receptor was expressed in *Xenopus* oocytes, it was shown to activate PLC (Ni *et al.*, 1997). In aggregate, the work of several laboratories has shown that the 5-HT$_{1A}$ receptor couples to PI-PLC in HeLa cells (Boddeke *et al.*, 1992; Harrington *et al.*, 1994; Middleton *et al.*, 1990), in Ltk$^-$ cells (Liu & Albert, 1991), in *Xenopus* oocytes (Ni *et al.*, 1997), and in BALB/c-3T3 cells (Abdel-Baset *et al.*, 1992), but only weakly or not at all in CHO cells (Cowen *et al.*, 1997; Newman-Tancredi *et al.*, 1992; Raymond *et al.*, 1992), Cos-7 cells (Fargin *et al.*, 1989), NIH-3T3 cells (Varrault *et al.*, 1992a), or GH_4C_1 cells (Liu & Albert, 1991).

Activation of protein kinase C (PKC) occurs in HeLa cells (Middleton *et al.*, 1990; Raymond *et al.*, 1989), and depends upon phospholipase C activation (Fargin *et al.*, 1989). This effect is probably mediated by G protein $\beta\gamma$ subunits, and almost certainly depends upon the expression of $\beta\gamma$-regulatable PLC. The coupling of the 5-HT$_{1A}$ receptor to PKC is as efficacious as that induced by the endogenous histamine receptor expressed in HeLa cells. The activation of PKC by the 5-HT$_{1A}$ receptor is relevant in that it increases Na^+-dependent phosphate uptake to a level similar to that induced by the endogenous histamine receptor (Raymond *et al.*, 1991). Thus, in HeLa cells, the 5-HT$_{1A}$ receptor regulates an endogenous transport process *via* PKC activation. However, like the activation phosphoinositide hydrolysis in HeLa cells, the coupling is much less efficient than is the coupling to the inhibition of adenylyl cyclase. Activation of PKC and Na^+-dependent phosphate uptake is more efficacious in cells expressing $\approx$3 pmol of receptors mg^{-1} protein than in cells expressing $\approx$500 fmol of receptors mg^{-1} protein (Raymond *et al.*, 1989).

The recombinant 5-HT$_{1A}$ receptor has also been shown to activate PLA_2 in HeLa cells (Harrington *et al.*, 1994), and to augment Ca^{2+}-induced arachidonic acid metabolism in CHO cells (Raymond *et al.*, 1992). Cowen *et al.* (1997) showed the 5-HT$_{1A}$ receptor activates phosphatidylcholine-specific phospholipase C (PC-PLC) in CHO cells. However, details of those linkages remain to be elucidated.

Regulation of channels

The recombinant 5-HT$_{1A}$ receptor has been shown to regulate the function of several distinct types of channels, including inwardly rectified K^+ channels, high conductance anion channels, CFTR Cl^- channels, and Ca^{2+} channels. G protein-gated inwardly rectified K^+ (GIRK) channels mediate hyperpolarizing postsynaptic potentials in the nervous system and in the heart during activation of $G_{i/oz}$-coupled receptors, including the 5-HT$_{1A}$ receptor (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Zgombick *et al.*, 1989). The regulation of GIRK channels by receptors relies upon the interaction of G protein $\beta\gamma$ subunits (released by receptor activation) with regulatory sites on the channels (Doupnik *et al.*, 1996). Karschin *et al.* (1991) used a highly efficient recombinant *vaccinia* virus vector system to express the 5-HT$_{1A}$ receptor in primary cultures of rat atrial myoctyes, and documented that the 5-HT$_{1A}$ receptor could stimulate an endogenous atrial inward rectifier K^+ current. Those studies were expanded by co-injecting rat atrial RNA with 5-HT$_{1A}$ receptor RNA into *Xenopus* oocytes (Dascal *et al.*, 1993), which resulted in the expression of a G protein-activated K^+ channel that could be activated by the 5-HT$_{1A}$ receptor. Moreover, the 5-HT$_{1A}$ receptor was also able to activate GIRK1 channels when receptor and channel RNAs were co-injected into *Xenopus* oocytes (Doupnik *et al.*, 1997).

One curious observation has been that the kinetical characteristics of GIRK regulation by receptors are markedly different in transfected cells *vs* in cells that natively express the GIRK channels and receptors. In neurons and atrial myocytes, the time courses for receptor-mediated GIRK current deactivation are 20–40 times faster than are those observed in systems in which cloned receptors and GIRK channels have been co-expressed heterologously (Andrade & Nicoll, 1986; Colino & Halliwell, 1987; Dascal *et al.*, 1993; Karschin *et al.*, 1991; Zgombick *et al.*, 1989). That finding suggested that additional components might be required to confer the rapid kinetical properties of the native transduction pathway. Doupnik *et al.* (1997) studied the effects of co-expression of the 5-HT$_{1A}$ receptor, GIRK1, and various 'regulators of G protein signalling' (RGS proteins) in *Xenopus* oocytes. They found that they could restore rapid activation and deactivation to GIRK current waveforms evoked by activation of 5-HT$_{1A}$ receptors by co-expression of RGS1, RGS3, or RGS4, but not by RGS2. This work provided evidence for functional regulation of 5-HT$_{1A}$ receptor-mediated GIRK activation by RGS1, RGS3, and RGS4.

The 5-HT$_{1A}$ receptor has been shown to regulate several other channels in transfected cells. Uezono *et al.* (1993) showed that the 5-HT$_{1A}$ receptor expressed in *Xenopus* oocytes could augment the activation of CFTR Cl^- channels induced by β_2-adrenoceptors. This effect was indirect in that the conditional activation of CFTR by the 5-HT$_{1A}$ receptor was enhanced by co-expression of adenylyl cyclase type II and G_{sz}, and likely proceeded *via* G protein $\beta\gamma$ subunits. Ni *et al.* (1997)

demonstrated that the 5-HT$_{1A}$ receptor expressed in *Xenopus* oocytes could also stimulate an oscillatory Ca^{2+}-activated Cl^- current. Mangel *et al.* (1993) showed that the 5-HT$_{1A}$ receptor inhibited a high conductance anion channel in CHO cells through either $G_{i\alpha2}$ or $G_{i\alpha3}$. The potential significance of the inhibition of high-conductance anion channels by the 5-HT$_{1A}$ receptor is not known, but those channels are thought to be important in cell volume regulation and the maintenance of membrane potential. Liu & Albert (1991) demonstrated that the rat 5-HT$_{1A}$ receptor inhibited Bay K8644-mediated Ca^{2+} influx in GH_4C_1 cells, and that this effect required expression of $G_{o\alpha}$ (Liu *et al.*, 1994). The ability of the 5-HT$_{1A}$ receptor to inhibit Ca^{2+} influx has been confirmed in a preliminary manner in several putative neuronal cell lines (NCB-20, F11, and HN2) (Singh *et al.*, 1996b). Thus, the 5-HT$_{1A}$ receptor can stimulate or inhibit multiple distinct ion channels in transfected cells.

Coupling to active ion transport processes

In addition to regulating channels, the 5-HT$_{1A}$ receptor has been shown to activate several active ion transport processes. In HeLa cells, the receptor stimulates Na^+-dependent phosphate uptake through a PKC-mediated pathway (Raymond *et al.*, 1989; 1990), and Na^+/K^+-ATPase through a Ca^{2+}-mediated pathway (Middleton *et al.*, 1990). Both pathways probably depend upon $G_{\beta\gamma}$-mediated stimulation of PLC. In CHO cells, the 5-HT$_{1A}$ receptor activates Na^+/H^+ exchange (Garnovskaya *et al.*, 1997; 1998) through a pathway that requires $G_{i\alpha}$, Src tyrosine kinase, and PI3-K, but not Mek, Ras, or Raf. The coupling to Na^+-dependent phosphate uptake suggests a potential role for the 5-HT$_{1A}$ receptor in regulating cellular energy processing, whereas the coupling to and Na^+/K^+-ATPase and Na^+/H^+ exchange suggests potential roles in cell volume regulation.

Coupling to G proteins

The ability of the 5-HT$_{1A}$ receptor to couple to various G protein subunits has been carefully studied using several different expression systems. Manning's group (Barr *et al.*, 1997; Butkerait *et al.*, 1995) used *Spodoptera frugiperda* (Sf9) cells for co-expression of the human 5-HT$_{1A}$ receptor with mammalian G protein subunits. They assessed receptor/G protein coupling by [^{35}S]-GTPγS binding and by guanine nucleotide-sensitive agonist binding assays. Co-expression of the receptor with members of the α_i group (but not others) together with various combinations of β_1 and γ subunits increased the affinity for agonists. Using a similar system, Mulheron *et al.* (1994) documented that the 5-HT$_{1A}$ receptor could also functionally couple to an endogenous G_o-like G protein in Sf9 cells. When the receptor was co-expressed with β_1 and γ_2, relatively equivalent coupling to α_{i1}, α_{i2}, α_{i3}, α_o and α_z was seen, whereas there was essentially no detectable coupling to α_{12}, α_{13}, α_s and α_q. When β_1 and α_{i1} were co-expressed with various γ-subunits, the following rank order of affinity was established: $\gamma_2 \approx \gamma_3 \approx \gamma_5 > \gamma_1$ (Barr *et al.*, 1997; Butkerait *et al.*, 1995).

Other groups have detected measurable differences in the affinity of 5-HT$_{1A}$ receptor for $G_{i/o/z}$ family α-subunits in various transfected cell systems. Clawges *et al.* (1997) added purified G protein subunits to Sf9 cell membranes expressing 5-HT$_{1A}$ receptors, and established that α_{i1}, α_{i2}, α_{i3}, and α_o were able to shift the receptors to a high-affinity state in the presence of either brain or retinal β_1/γ. α-Transducin ($G_{t\alpha}$) subunits were inactive regardless of which β_1/γ preparation was used. A significantly higher affinity for agonist was observed for the 5-HT$_{1A}$ receptor in the presence of α_{i3} compared with either α_{i2} or α_o. Bertin *et al.* (1992) expressed 5-HT$_{1A}$ receptors in *E. coli* to determine a relative rank order of affinity for this receptor to reconstituted purified mammalian G protein α-subunits of $G_{i\alpha3} > G_{i\alpha2} > G_{i\alpha1} \gg G_{o\alpha} \gg G_{s\alpha}$. Another group (Garnovskaya *et al.*, 1997; Raymond *et al.*, 1993b) demonstrated agonist-promotable physical coupling of the 5-HT$_{1A}$ receptor to G proteins in HeLa and CHO cells using high affinity agonist binding and co-immunoprecipitation assays. Agonist treatment induced coupling of the 5-HT$_{1A}$ receptors to G proteins with an apparent rank order of $G_{i\alpha3} > G_{i\alpha2} \approx G_{i\alpha1} \approx G_{o\alpha} > G_{z\alpha} \gg G_{s\alpha}$. Thus, despite using markedly different transfections systems (bacteria, insect and mammalian cells) there is reasonable consensus that the 5-HT$_{1A}$ receptor functionally couples nearly exclusively through α-subunits of $G_{i/o/z}$ proteins, with a likely rank order of $G_{i\alpha3} > G_{i\alpha2} \geqslant G_{i\alpha1} \geqslant G_{o\alpha} > G_{z\alpha}$. There is currently little evidence that the 5-HT$_{1A}$ receptor can couple to $G_{t\alpha}$, $G_{s\alpha}$, $G_{q\alpha}$, $G_{12\alpha}$ or $G_{13\alpha}$.

Some groups have taken these studies a step further by trying to link specific G protein subunits with signals that occur downstream of the G proteins. Fargin *et al.* (1991) used an immuno-neutralization method to link $G_{i\alpha3}$ to the inhibition of adenylyl cyclase and to the activation of PI-PLC in HeLa cells. Raymond *et al.* (1993b) used a similar method in CHO and HeLa cells to demonstrate that 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase can be mediated by either $G_{i\alpha3}$ or $G_{i\alpha2}$, and probably $G_{i\alpha1}$. These findings are not surprising in light of the work of Wong *et al.* (1992) that demonstrated that $G_{i\alpha1}$, $G_{i\alpha2}$, $G_{i\alpha3}$, and $G_{z\alpha}$, can all inhibit cyclic AMP accumulation in mammalian cells. Gettys *et al.* (1994) used selective photoaffinity labelling of $G_{i\alpha}$ subunits in CHO cells to demonstrate a close correlation between the ability of a panel of 5-HT$_{1A}$ receptor agonists to activate $G_{i\alpha2}$ and to inhibit adenylyl cyclase, whereas there was much less correlation between $G_{i\alpha3}$ and the inhibition of adenylyl cyclase. Immunoneutralization studies suggested that $G_{i\alpha3}$ and $G_{i\alpha2}$ were about equally important for the inhibition of adenylyl cyclase in HeLa cells, whereas $G_{i\alpha2}$ was more important in CHO cells. The differences in the relative importance of $G_{i\alpha2}$ and $G_{i\alpha3}$ may have been affected by the relative abundance of the G protein α-subunits in HeLa and CHO cells. In HeLa cells, $G_{i\alpha3}$ is much more highly expressed than $G_{i\alpha2}$ (which is almost undetectable by immunoblot), whereas in CHO cells, there is $\approx$9 fold more $G_{i\alpha2}$ than $G_{i\alpha3}$ (Raymond *et al.*, 1993b).

Liu *et al.* (1994) used an elegant antisense approach to show that $G_{i\alpha2}$ was primarily responsible for 5-HT$_{1A}$ receptor-mediated inhibition of adenylyl cyclase in GH_4C_1 cells, whereas $G_{o\alpha}$ was responsible for the inhibition of Ca^{2+} channels. Using the same system, they have also shown that $G_{i\alpha2}$ is responsible for 5-HT$_{1A}$ receptor-mediated increases in intracellular Ca^{2+} in Ltk^- cells (Albert *et al.*, 1996). Garnovskaya *et al.* (1997) showed that either $G_{i\alpha2}$ or $G_{i\alpha3}$ could couple the 5-HT$_{1A}$ receptor to the activation of Na^+/H^+ exchange in CHO cells, whilst $G_{i\alpha1}$, $G_{o\alpha}$, and $G_{z\alpha}$ could not. Langlois *et al.* (1996) presented evidence to suggest that 5-HT$_{1A}$ receptors inhibit adenylyl cyclase *via* $G_{i\alpha3}$ on the apical cell surface, and *via* $G_{i\alpha2}$ on the basolateral surface of polarized epithelial LLC-PK$_1$ cells. In aggregate, these studies link the effects of the 5-HT$_{1A}$ receptor to the inhibition of adenylyl cyclase, activation of Na^+/H^+ exchange, and activation of PLC through $G_{i\alpha2}$ or $G_{i\alpha3}$. The inhibition of Ca^{2+} channels appears to require $G_{o\alpha}$.

DNA synthesis, growth, and transformation

5-HT receptors coupled to pertussis toxin-sensitive G proteins have previously been implicated as growth stimulatory (Ishizuka *et al.*, 1992; Seuwen *et al.*, 1988). Furthermore, in glial cells, endogenous 5-HT$_{1A}$ receptors stimulate secretion of the S100 protein, which has been shown to promote the growth of serotoninergic neurons (Lauder, 1993; Whitaker-Azmitia, 1991). However, a direct link between the 5-HT$_{1A}$ receptor and growth cascades has not been demonstrated in cells in which the receptor is expressed naturally. Therefore, three different transfected cell lines have been used to study the links of 5-HT$_{1A}$ receptors to DNA synthesis, growth and cellular transformation. Abdel-Baset *et al.* (1992) transfected BALB/c-3T3 fibroblasts with the rat 5-HT$_{1A}$ receptor, and showed that 5-HT induced enhanced incorporation of [^{3}H]-thymidine into DNA in a clone that expressed 600 fmol mg^{-1} of protein of [^{3}H]-8-OH-DPAT. Long-term treatment of cell cultures resulted in phenotypical transformation and foci formation in transfected, but not in non-transfected cells. Those effects were sensitive to pertussis toxin, and were attributed to the actions of PI-PLC. Another group contemporaneously showed that the 5-HT$_{1A}$ receptor expressed in NIH-3T3 cells (at 40–500 fmol mg^{-1} of protein) induced focus formation, weakly stimulated DNA synthesis, and potentiated increases in DNA synthesis initiated by epidermal growth factor (Varrault *et al.*, 1992a). Those effects were also sensitive to pertussis toxin, and did not appear to involve PI-PLC or lowering of intracellular cyclic AMP levels. Lam *et al.* (1996) showed that the 5-HT$_{1A}$ receptor expressed in Rat-1 fibroblasts increased proliferation as measured by the R-SAT method (Messier *et al.*, 1995). Thus, the 5-HT$_{1A}$ receptor can stimulate proliferation and/or transformation in several transfected cell types as measured by different methods.

Regulation of transcriptional cascades

The 5-HT$_{1A}$ receptor has been linked to two specific signalling pathways involved in the regulation of transcription, namely activation of Erk (extracellular signal-regulated kinase) family mitogen-activated protein kinases, and the transcriptional regulatory factor, NF-κB (nuclear factor-κB). Cowen *et al.* (1997) used transfected CHO cells to document that 5-HT$_{1A}$ receptor agonists activate a signalling pathway that stimulates NF-κB, probably through accelerating the degradation of an inhibitor of NF-κB called IκBα. Phosphorylation of IκBα causes dissociation from NF-κB, resulting in nuclear translocation of NFκ-B, and accelerated degradation of IκBα *via* ubiquitination (Berg & Baldwin, 1993; Brown *et al.*, 1993). Cowen *et al.* (1997) also showed that the degradation of IκBα was sensitive to inhibition of phosphatidylcholine-specific phospholipase C (PC-PLC).

Several groups have documented that the 5-HT$_{1A}$ receptor activates Erk kinases in CHO cells, and have identified a number of signalling molecules involved in that pathway. Like other G protein-coupled receptors (Luttrell *et al.*, 1997; Marshall, 1995), the 5-HT$_{1A}$ receptor activates Erk through a complex pathway that involves many of the same molecules used by growth factor receptor tyrosine kinases. The activation of Erk by the 5-HT$_{1A}$ receptor is initiated by $\beta\gamma$ subunits released from pertussis toxin-sensitive G proteins. This results in tyrosine phosphorylation of Shc, a protein that serves as a docking platform. Shc phosphorylation results in the recruitment of a lipid kinase, and an adapter protein, Grb2 to the signalling complex. Grb2, in turn, binds to a Ras activator protein called Sos. Ras activation leads to sequential activation of Raf, which phosphorylates and activates MEK (mitogen and extracellular signal regulated kinase), which phosphorylates and activates Erk (Garnovskaya *et al.*, 1996). The precise role of PI3-K in propagating the Erk signal is not known, but it is clearly necessary for 5-HT$_{1A}$ receptor-mediated Erk activation, and most likely operates at the level of Shc and Grb2 (Cowen *et al.*, 1996; Garnovskaya *et al.*, 1996; 1998). More recent studies have also implicated a Ca^{2+}/calmodulin-dependent endocytosis step between Ras and Raf (Della Rocca *et al.*, 1999), and reactive oxygen intermediates upstream of Src in 5-HT$_{1A}$ receptor-mediated ERK activation in CHO cells (Mukhin, Raymond and Garnovskaya, unpublished observation). An NAD(P)H oxidase enzyme probably produces the reactive oxygen species.

Cowen *et al.* (1996) hypothesized a role for phosphatidylcholine-specific PLC (PC-PLC) in 5-HT$_{1A}$ receptor-mediated Erk activation based on their observations that 8-OH-DPAT elicited release of radioactivity from cells pre-loaded with [^{3}H]-choline. This effect and Erk activation were both attenuated by a PC-PLC inhibitor (tricyclodecan-9-yl-xanthogenate, D609). If PC-PLC is required for 5-HT$_{1A}$ receptor-mediated Erk activation in CHO cells, it most likely functions at or upstream of Raf based on several studies in other systems. Introduction of bacterial PC-PLC activates Raf, and D609 blocks Raf activation induced by epidermal growth factor and serum in NIH-3T3 cells. Dominant negative Raf constructs block replication, and this block is not overcome by introduction of bacterial PC-PLC (Cai *et al.*, 1992; 1993). Moreover, dominant negative Ras attenuates the activation of PC-PLC in NIH-3T3 cells (Cai *et al.*, 1993). Based on these studies and their own work, Cowen *et al.* (1996) hypothesized that PC-PLC augments the activation of Raf that is induced by Ras. If this were so, it would appear that the 5-HT$_{1A}$ receptor in CHO cells activates Erk through the activation of separate, yet converging lipid signalling pathways. The first involves PI3-K and intersects the Erk pathway upstream of Ras, whereas the second involves PC-PLC and also intersects the Erk pathway upstream of Ras.

Desensitization and phosphorylation

Desensitization is a process through which signalling pathways become progressively less able to mount a signal after repeated exposures to a stimulus. One major mechanism through which desensitization of G protein-coupled receptors occurs is kinase-directed phosphorylation of the receptors. Thus far, three distinct protein kinases have been implicated in the desensitization and phosphorylation of the 5-HT$_{1A}$ receptor, namely PKC, PKA, and GRK (G protein-coupled receptor kinase). There are four putative PKC sites and two putative PKA sites in the human 5-HT$_{1A}$ receptor (see Figure 1 and its legend). Stimulation of PKC by application of phorbol esters induces a rapid phosphorylation of the receptor at a stoichiometry of two phosphates per receptor (Raymond, 1991). This phosphorylation is associated with desensitization of several signals in various cell lines. In CHO cells, PKC-mediated desensitization of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor is manifested only by a change in potency of agonist, and is not associated with a significant change in efficacy (Raymond, 1991), whereas in P11 rat pituitary cells, PKC-mediated desensitization is manifested by a change in both and efficacy of 8-OH-DPAT to cause inhibition of adenylyl cyclase (Hensler *et al.*, 1996). In contrast, phorbol ester pretreatment of Ltk$^-$ fibroblasts and

GH_4C_1 cells transfected with the 5-HT$_{1A}$ receptor has no detectable effects on the inhibition of adenylyl cyclase (Lembo *et al.*, 1995; 1997; Liu & Albert, 1991). In Ltk$^-$ fibroblasts, activation of PKC with phorbol ester results in desensitization of 5-HT$_{1A}$ receptor-mediated PLC activity as measured by increased intracellular Ca^{2+} and hydrolysis of phosphoinositides. The effects of phorbol ester on Ca^{2+} mobilization were profound, essentially eliminating subsequent responses to 5-HT. Desensitization could be reversed by mutation of three putative PKC sites in the i3 loop of the 5-HT$_{1A}$ receptor (Lembo *et al.*, 1995), providing strong support for a functional link between phosphorylation and desensitization of the receptor. Harrington *et al.* (1994) provided further evidence for PKC-mediated desensitization of the 5-HT$_{1A}$ receptor. They showed that pretreatment with phorbol ester of 5-HT$_{1A}$-receptor transfected HeLa cells rapidly uncoupled the receptor from G proteins, and that inhibitors of PKC could block this effect. Thus, the evidence is fairly clear that PKC can induce desensitization and phosphorylation of the 5-HT$_{1A}$ receptor in transfected cells, although the functional consequences of the interaction varies depending on the cell type and signalling pathways being measured.

There is also strong evidence that activation of PKA can lead to phosphorylation and desensitization of the 5-HT$_{1A}$ receptor in multiple cell types. In CHO cells, PKA stimulation leads to phosphorylation of the receptor with a stoichiometry

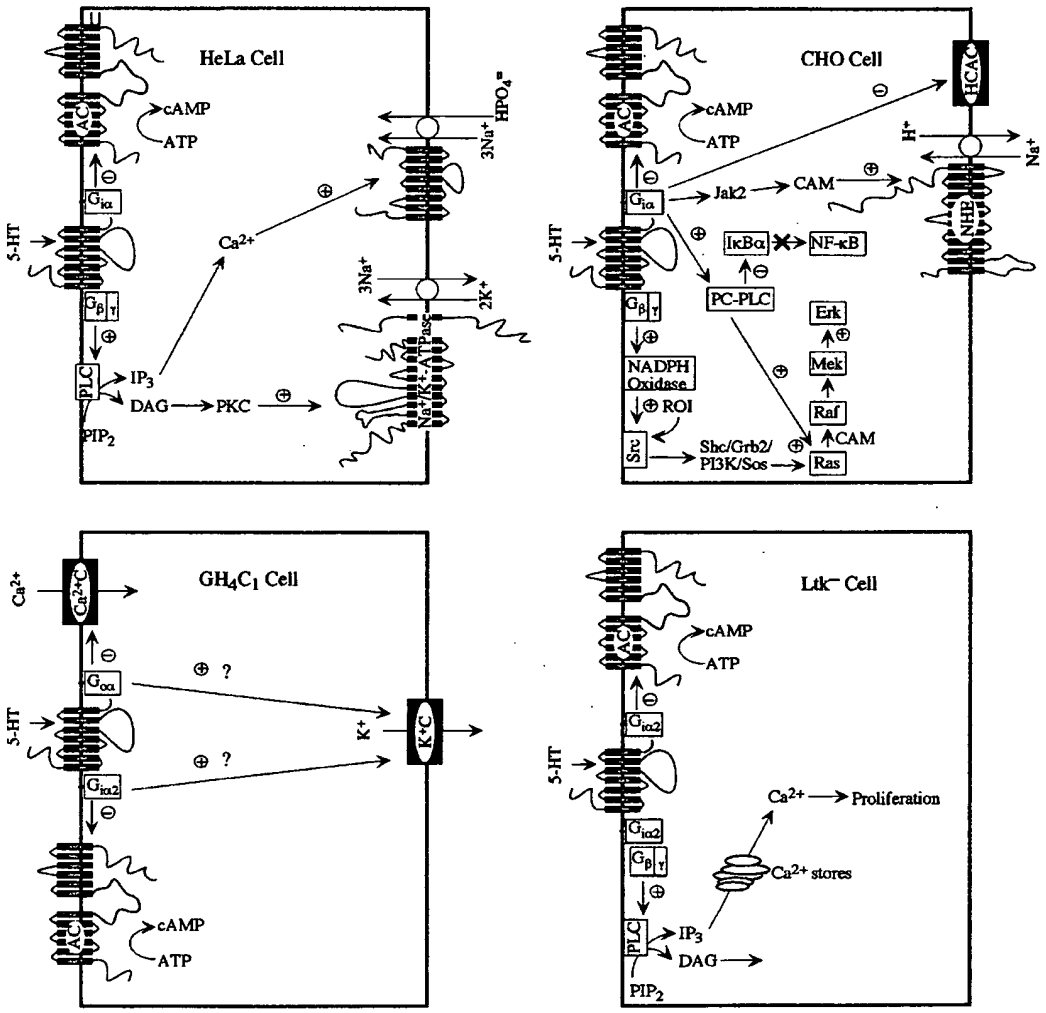

Figure 2 Second messenger and effector linkages of the 5-HT$_{1A}$ receptor in transfected cell systems. There are differences in the specific linkages depending upon the host cell in which the receptor has been expressed. Depicted are 5-HT$_{1A}$ receptor linkages with the inhibition of adenylyl cyclase (AC), stimulation of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis, increased levels of intracellular Ca^{2+}, and activtion of phospholipases A_2 (PLA_2) and C (PLC). Phosphatidylinositol-specific PLC and phosphatidylcholine-specific PLC are abbreviated as (PI-PLC and PC-PLC). The receptor also couples to protein kinase C (PKC), activation of K^+ channels, inhibition of high conductance anion channels (HCAC) and Ca^{2+} channels, and activation of Erk extracellular signal regulated protein kinase. Erk activation proceeds from Src tyrosine kinase to a docking platform (Shc), where PI-3K (PI-3′ kinase) Sos (a G protein activator) and Grb2 (an adapter) aggregate. Sos activates the small monomeric G, Ras. This activates a sequential phosphorylation cascade involving Raf, Mek (mitogen and extracellular signal regulated protein kinase), and Erk. Erk activation also requires calmodulin (CAM) and reactive oxygen intermediates (ROI) generated by an NAD(P)H oxidase. The receptor couples to active transporters in CHO cells, including the Na^+/H^+-exchanger, Na^+/K^+-ATPase and Na^+/PO_4 cotransporter. All linkages described are sensitive to pertussis toxin, and involve either G_i or G_o proteins. α, β, and γ represent subunits of G proteins. Other abbreviations used are IP_3 (inositol 1,4,5-trisphosphate), and DAG (diacylglycerol).

of one phosphate per receptor. Pharmacological stimulation of PKA also augments PKC-induced phosphorylation and desensitization of the inhibition of adenylyl cyclase by the 5-HT$_{1A}$ receptor (Raymond & Olsen, 1994). Those results confirmed the observations of Liu & Albert (1991), who first showed that activators of PKA enhanced the phorbol ester-induced desensitization of 5-HT$_{1A}$ receptor coupling to PLC in Ltk$^-$ fibroblasts. In HeLa cells, Harrington *et al.* (1994) documented rapid PKA-induced reduction in high affinity agonist binding as well as a total attenuation of the ability of the 5-HT$_{1A}$ receptor to inhibit adenylyl cyclase.

The 5-HT$_{1A}$ receptor can mediate its own desensitization. Prolonged treatment with 5-HT leads to downregulation and desensitization of 5-HT$_{1A}$ receptors in Swiss 3T3 cells (van Huizen *et al.*, 1993). In HeLa cells, treatment with 8-OH-DPAT leads to rapid uncoupling of the receptor from G proteins and from the inhibition of adenylyl cyclase. These effects appear to depend upon PKC, PLA$_2$, and Ca^{2+} (Harrington *et al.*, 1994). In P11 cells, pretreatment with the agonist 5-carboxamidotryptamine results in a rightward shift of the concentration-response curve of 8-OH-DPAT-inhibited adenylyl cyclase activity (Hensler *et al.*, 1996). In Sf9 insect cells, pretreatment with 5-HT leads to rapid phosphorylation of the 5-HT$_{1A}$ receptor on serine and threonine residues, and to uncoupling of the receptor from G proteins and from the inhibition of adenylyl cyclase (Nebigil *et al.*, 1995). This effect could be blocked by heparin, but not by inhibitors of PKC, and was thus attributed to the actions of a GRK. There are 17 serine and threonine residues on the intracellular loops that might serve as potential GRK phosphorylation sites.

Correlation of receptor structure and function

Despite being one of the first G protein coupled receptors to be cloned, relatively few detailed structure-function studies have been performed on the 5-HT$_{1A}$ receptor. One of the major distinctive pharmacological characteristics of the 5-HT$_{1A}$ receptor is a high affinity for classical β-adrenoceptor blockers such as pindolol. Guan *et al.* (1992) delineated a single residue critical in the binding of β-adrenoceptor blockers to the 5-HT$_{1A}$ receptor. They mutated Asn^{386} in the seventh transmembrane domain of the human 5-HT$_{1A}$ receptor, based on the observation that this residue is uniquely conserved in all 5-HT$_{1A}$ and β-adrenoceptors of different species. Mutation of this residue to valine caused a selective decrease in the affinity of pindolol and similar ligands for the mutant Asn^{386}→Val receptor, whilst producing insignificant changes in the binding of other 5-HT$_{1A}$ receptor ligands. Thus, Asn^{386} is critical for binding to β-adrenoceptor blockers like pindolol, but not to 5-HT. Kuipers *et al.* (1997) examined the effects of the same mutation on a range of aryloxypropanolamine enantiomers and further suggested that Asn^{386} functions as a chiral discriminator in that the Asn^{386}→Val mutation more significantly lowered the affinities of the S-enantiomers in a ligand binding assay.

Ho *et al.* (1992b) used a *vaccinia* infection-transfection method to transiently express wild-type and mutant 5-HT$_{1A}$ receptors into COS-7 cells in order to study the effects of various point mutations in putative transmembrane regions on receptor ligand binding. Three substitutions, Asp^{82}→Asn, Asp^{116}→Asn, and Ser^{199}→Ala, resulted in a 60–100 fold decreased affinity of 5-HT for the receptor, but had no effect on the affinity of the antagonist, pindolol. The binding of 5-HT to a fourth mutant, Thr^{200}→Ala, was not measurable. Nevertheless, 5-HT induced GTPase activities for all of the mutant receptors studied. These findings indicate that Asp^{82}, Asp^{116}, and Ser^{199} play important roles in the binding of 5-HT, but have little effect on pindolol binding. Thr^{200} is important in binding to both 5-HT and to pindolol. By analogy with the β-adrenoceptor, Asp^{82} and/or Asp^{116} are likely to act as a counterion for the amine group of 5-HT (Strader *et al.*, 1989). While the first acidic residue is conserved in all cloned G protein-coupled receptors reported thus far, the second one is present in only the receptors which bind the bioamines epinephrine, norepinephrine, dopamine, acetylcholine and serotonin. These findings have been nicely integrated into a model of the 5-HT$_{1A}$ receptor pharmacophore by Kuipers *et al.* (1994).

Varrault *et al.* (1994) used a different approach to study potential G protein contact sites within the 5-HT$_{1A}$ receptor. They constructed synthetic peptides derived from the second (i2) and third (i3) intracellular loops of the human 5-HT$_{1A}$ receptor, and assessed the ability of those peptides to modulate the binding of a nonhydrolysable GTP analogue to G$_{i/o}$, and to inhibit adenylyl cyclase. A peptide consisting of the entire i2 loop (Asp^{133}-Arg^{153}) and a heptadecapeptide from the carboxyl terminal region of the i3 loop (Ala^{331}-Leu^{347}), but not a nonapeptide from the carboxyl terminal region of the i3 loop (Ala^{336}-Val^{344}), inhibited forskolin-stimulated adenylyl cyclase activity in membranes derived from NIH-3T3 cells, S49 cells, and rat hippocampus, and increased GTPγS binding to purified bovine brain G$_{i/o}$ proteins. Thus, those studies identified the entire i2 loop and a carboxyl terminal heptadecapeptide of the i3 loop of the human 5-HT$_{1A}$ receptor as key G protein regulatory sites. These sequences contain key threonine residues shown to be involved in receptor desensitization and signal transduction by Lembo *et al.* (1995; 1997). More recently, Albert *et al.* (1998) presented evidence that supported a key role for Thr^{149} in the i2 loop of the rat receptor in coupling specifically to G$_{\beta\gamma}$-mediated signals. These studies support a model in which the i2 loop and portions of the i3 loop form amphipathic α-helices aligned such that a hydrophobic G protein interaction site is formed, as described by Albert *et al.* (1998).

Lembo *et al.* (1995; 1997) used site-directed mutagenesis to examine the roles of intracellular threonine and serine residues in the i2 and i3 loops of the rat 5-HT$_{1A}$ receptor. They found that four specific residues played roles in signal initiation, and also in PKC-mediated desensitization of the receptor. A detailed mutagenesis study of the PKC sites on the receptor was needed because PKC-mediated phosphorylation reactions could affect the function of the receptor (Raymond, 1991), G$_i$ proteins (Drummond, 1985; Daniel-Issakani *et al.*, 1989; Bushfield *et al.*, 1990; Yatomi *et al.*, 1992; Strassheim & Malbon, 1994) or PLC (Ryu *et al.*, 1990; Ali *et al.*, 1997), any of which effects could lead to desensitization of this pathway. By constructing and expressing several mutant receptors with conservative point mutations, they showed that two threonines and one serine residue located within consensus PKC phosphorylation sequences in the i3 loop were needed to confer PKC-mediated desensitization of Ca^{2+} mobilization in Ltk$^-$ fibroblasts. When the individual point mutations (Thr^{229}→Ala, S^{253}→Gly, and Thre^{343}→A) were tested, there was no difference in desensitization when compared with non-mutated receptors. In contrast a double mutant (Thr^{229}→Ala/S^{253}→Gly) and a triple mutant (Thr^{229}→Ala/S^{253}→Gly/Thre^{343}→Ala) became progressively more resistant to PKC-mediated desensitization. Those results suggest that there is a good correlation between the presence of those three residues and PKC-mediated desensitization of the 5-HT$_{1A}$ receptor.

Lembo *et al.* (1997) also assessed the role of Thr^{149} in signal transduction by expressing a mutant rat 5-HT$_{1A}$ receptor (Thr^{149}→Ala) in Ltk$^-$ fibroblasts and in GH_4C_1 cells. They found that the mutant receptor lost its ability to elevate intracellular Ca^{2+} in Ltk$^-$ cells, and also was unable to inhibit opening of BayK8644 sensitive Ca^{2+} channels in GH_4C_1 cells. In contrast, the Thr^{149}→Ala mutation only partially uncoupled the receptor from adenylyl cyclase inhibition. Interestingly, the Thr^{229}→Ala mutation also partially uncoupled the receptor from the inhibition of adenylyl cyclase, and reduced the peak increase in intracellular Ca^{2+} in Ltk$^-$ cells (Lembo *et al.*, 1995). Thus, the work of Lembo *et al.* (1995; 1997) suggests those three residues in the i3 loop of the 5-HT$_{1A}$ receptor (Thr^{229}, S^{253}, and Thre^{343}) mediated PKC-induced desensitization of the rat receptor. Additionally, Thr^{229} is important for efficient coupling to both inhibition of adenylyl cyclase and elevations of intracellular Ca^{2+}. Thr^{149} in the i2 loop is highly critical in both elevations of intracellular Ca^{2+} and blockade of Ca^{2+} channels by the receptor, and plays a smaller, but significant role in the inhibition of adenylyl cyclase.

Allelic variants of the 5-HT$_{1A}$ receptor

The possibility that the 5-HT$_{1A}$ receptor might possess allelic variants was supported by the cloning of the rat receptor by two different groups (Albert *et al.*, 1990; Fujiwara *et al.*, 1990) whose published nucleic acid sequences differed only by two nucleotides. One of the variants was silent at the protein level, whereas the other resulted in a predicted change in a single amino acid in the rat 5-HT$_{1A}$ receptor (Fujiwara *et al.*, 1993). Several groups have subsequently described variations in the 5'-untranslated and the coding regions of the human 5-HT$_{1A}$ receptor gene. Some of the nucleic acid changes in the coding block do not result in amino acid changes, whereas several others result in single amino acid changes; these include Pro^{16}→Leu, Ile^{28}→Val, Gly^{22}→Ser, Arg^{219}→Leu; Gly^{272}→Asp; Asn^{417}→Lys (Erdmann *et al.*, 1995; Harada *et al.*, 1996; Kawanishi *et al.*, 1998; Lam *et al.*, 1996; Nakhai *et al.*, 1995). The existence of these variant sequences raises the interesting possibility that there may be functional differences that could lead to disease manifestations. Although a detailed biochemical analysis of each of the variants has not yet been performed, Brüss *et al.* (1995) studied the Ile^{28}→Val variant in transfected COS-7 cells. They demonstrated that this receptor variant had ligand binding properties that were nearly identical to those of the wild-type receptor (Brüss *et al.*, 1995). In addition, to this point, no definitive links in populations between the allelic variants have been made to alcoholism, schizophrenia, bipolar affective disorder, or Tourette's syndrome.

Ligand pharmacology

Cells transfected with the 5-HT$_{1A}$ receptor could provide a ready source for the study of this receptor absent of any input from other 5-HT receptors. However, the usefulness of pharmacological information obtained from transfected cell systems required some measure of validation. Several groups have directly compared the ligand binding and second messenger coupling parameters of transfected 5-HT$_{1A}$ receptors with those naturally expressed in tissues. They have concluded that transfected cell systems are valid for studying the pharmacology of the 5-HT$_{1A}$ receptor (Pauwels *et al.*, 1993; Pou *et al.*, 1997). Transfected cell lines have been used to characterize radioligands (Sundaram *et al.*, 1992; 1995) and identify and characterize partial agonists (Arthur *et al.*, 1993; Assie *et al.*, 1997; Pauwels *et al.*, 1993; 1997) and inverse agonists (Barr & Manning, 1997; Newman-Tancredi *et al.*, 1998) of the 5-HT$_{1A}$ receptor. They have also been used in the development of silent antagonists of the 5-HT$_{1A}$ receptor, which has been a particularly nettlesome task because most putative silent antagonists actually turned out to be partial agonists (Routledge, 1996). Finally, WAY-100635, (N-[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-n-(2-pyridiyl) cyclohexanecarboxamide trihydrochloride) was characterized as a silent antagonist in a number of *in vivo* and *in vitro* models (Fletcher *et al.*, 1996). Critically, the properties of WAY-100635 as a true silent antagonist of the 5-HT$_{1A}$ receptor were confirmed in a transfected CHO cell system using GTPγS binding (Newman-Tancredi *et al.*, 1996) and measurements of the metabolic production of protons (Dunlop *et al.*, 1998).

Transfected cells have also been used to characterize the interactions of novel ligands with the 5-HT$_{1A}$ receptor. For example, transfected cells were instrumental in delineating the specific interactions of modified oleamide, an endogenous sleep-associated fatty acid primary amide, with 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Boger *et al.*, 1998). Transfected cells were also used to disprove an interaction between 5-HT-moduline (Leu-Ser-Ala-Leu) and the 5-HT$_{1A}$ receptor and to confirm its interaction specifically with a very high apparent affinity and in a non-competitive manner with 5-HT$_{1B}$ receptors (Rousselle *et al.*, 1998).

Coupling efficiency

Transfected cell systems have been used to compare the efficiency of coupling of the 5-HT$_{1A}$ receptor to various second messengers and G proteins. For example, the 5-HT$_{1A}$ receptor expressed in HeLa cells both inhibits adenylyl cyclase and activates PLC (Fargin *et al.*, 1989). However, the receptor has been shown to differentially modulate those activities. The 5-HT$_{1A}$ receptor couples very efficiently to the inhibition of adenylyl cyclase because changing receptor expression levels from <20 fmol mg^{-1} protein to ≈ 3 pmol mg^{-1} of protein had no effect on the potency or efficacy of agonists to inhibit cyclic AMP accumulation (Boddeke *et al.*, 1992; Fenrick *et al.*, 1996). This tight coupling applied over a variety of full and partial agonists (Schoeffter *et al.*, 1996). In contrast, coupling of the 5-HT$_{1A}$ receptor to PLC as measured by Ca^{2+} mobilization and phosphoinositide hydrolysis was much less efficient in that the potency, and especially the efficacy, of various agonists was considerably less than for the inhibition of adenylyl cyclase. Moreover, both efficacy and potency of coupling of the 5-HT$_{1A}$ receptor to PLC were improved at higher levels of receptor expression (Boddeke *et al.*, 1992; Fargin *et al.*, 1989; Fenrick *et al.*, 1996; Raymond *et al.*, 1989; Schoeffter *et al.*, 1997).

Transfected cells have been used to study other aspects of 5-HT$_{1A}$ receptor coupling efficiency, including variables that affect ligand efficacy and potency. One such variable is receptor/G protein stoichiometry. Newman-Tancredi *et al.* (1997) studied the effects of altering receptor : G protein ratios in CHO cells by examining [^{35}S]-GTPγS binding in transfected lines bearing various numbers of 5-HT$_{1A}$ receptors. Their data suggested that increasing receptor/G-protein ratio (i) augments the potency of full agonists, (ii) increases the efficacy of partial agonists and (iii) increases the negative efficacy of inverse agonists at recombinant human 5-HT$_{1A}$ receptors. Those conclusions were similar to those of Boddeke *et al.* (1992),

who showed that increasing receptor numbers were associated with higher efficacy of partial agonists to stimulate PLC in HeLa cells. The levels of G proteins were constant among the cell lines in those two studies, whereas receptor levels varied. Raymond *et al.* (1992) altered the ratios of 5-HT$_{1A}$ receptors to G proteins by treating CHO cells with varying amounts of pertussis toxin to neutralize fractions of endogenous G proteins. They showed that increasing amounts of pertussis toxin resulted first in a rightward shift of the IC_{50} of 5-HT for the inhibition of adenylyl cyclase (reduction in potency) followed by a reduction in the efficacy. Those results are consistent with either the classical or operational models of receptor/effector coupling (Black *et al.*, 1985; Kenakin & Morgan, 1989), both of which models assume unconstrained interactions of the various signalling molecules. In contrast, Varrault *et al.* (1992b) used transfected NIH-3T3 cells to show that increasing 5-HT$_{1A}$ receptor/G protein stoichiometry mainly increased the efficacies, but not the potencies of various full and partial agonists to inhibit adenylyl cyclase. In a few cases, alterations in efficacy were seen, but these were less than predicted, and varied depending upon the specific ligands being studied (Varrault & Bockaert, 1992). These latter results are consistent with a constrained model of receptor/G protein interaction in which the ability to interact efficiently is damped by functional membrane compartmentalization. The findings are significant in aggregate as they suggest that cell-specific constraints may alter the effects of 5-HT$_{1A}$ receptor/G protein coupling on ligand efficacy in various second messenger pathways.

Another variable that could affect ligand efficacy and potency at the 5-HT$_{1A}$ receptor is the possibility that certain ligands induce conformational changes in the receptor that are more or less favourable for G protein activation. For example, partial agonists could be less efficacious than full agonists because they are unable to induce the optimal conformational change in the receptor that regulates contact with G proteins. Gettys *et al.* (1994) confirmed that full agonists of the 5-HT$_{1A}$ receptor were more efficacious in activating $G_{i\alpha}$ subunits in CHO cells than were partial agonists. They further tested the hypothesis that partial agonists might induce relatively selective activation of specific $G_{i\alpha}$ subunits when compared with full agonists. They provided evidence in CHO cells that $G_{i\alpha 3}$ was activated with a rank order of efficacy of 8-OH-DPAT $\approx$ 5-HT > ipsapirone $\approx$ rauwolscine, whereas $G_{i\alpha 2}$ was activated with a rank order of efficacy of 5-HT > 8-OH-DPAT > rauwolscine > ipsapirone. Thus, both receptor/G protein stoichiometry and the availability of specific isotypes of $G_{i\alpha}$ subunits might affect the coupling efficiency of the 5-HT$_{1A}$ receptor to G proteins and specific second messengers.

Transcriptional regulation of the 5-HT$_{1A}$ receptor

Variables that regulate the absolute levels and the stability of receptor mRNA probably lead to critical regulation of receptor protein expression and function. For the 5-HT$_{1A}$ receptor, this point was experimentally tested by Konigs *et al.* (1995), who showed that levels of receptor mRNA (as measured by Northern blot and *in situ* hybridization) correlated very well with receptor protein expression (as measured by radioligand binding) in clones of Swiss 3T3 cells transfected with the human 5-HT$_{1A}$ receptor. The levels of expression of the 5-HT$_{1A}$ receptor can be altered *in vivo* by several distinct stimuli. For example, glucocorticoids have been shown to negatively regulate 5-HT$_{1A}$ receptor mRNA expression within the hippocampus (Chalmers *et al.*, 1994). The selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT differentially regulates the levels of 5-HT$_{1A}$ receptor mRNA in the dorsal and median raphe nuclei (Razani *et al.*, 1997). Hyperammonaemia increased 5-HT$_{1A}$ receptor mRNA and protein expression in rat hippocampus and transfected HN2 cells (Alexander *et al.*, 1995). Stress induced by serum deprivation increases 5-HT$_{1A}$ receptor protein and message levels in transfected HN2 and NCB-20 cells (Singh *et al.*, 1996b). The recombinant 5-HT$_{1A}$ receptor also has been shown to modulate the levels of gangliosides when expressed in HN2 hippocampal, dorsal root ganglion-derived F-11 cells and NCB-20 brain cells (Singh *et al.*, 1996a). This may represent a cellular defense response in that 5-HT$_{1A}$ receptors protected primary cultures of neurons from chick embryo telencephalons from apoptotic cell death, possibly by stimulating production of NGF (Ahlmeyer & Kriegelstein, 1997). Despite the potential importance of the regulation of 5-HT$_{1A}$ receptor message and protein levels, almost nothing is known about the molecular mechanisms through which those various stimuli regulate the expression of the 5-HT$_{1A}$ receptor. In that regard, Charest *et al.* (1993) have identified a septal cell line that can be induced to express 5-HT$_{1A}$ receptors by retinoic acid treatment. Other non-neuronal cell lines (Jurkat cells) have also been suggested to express the 5-HT$_{1A}$ receptor (Aune *et al.*, 1993; 1994). These cell lines might prove to be useful for analysis of promoter elements of the 5-HT$_{1A}$ receptor gene that regulate its expression in the central nervous system.

Conclusions

The isolation of the gene and cDNA for the 5-HT$_{1A}$ receptor has resulted in an explosion of information regarding the characteristics and functions of this receptor in heterologous expression systems. This information has translated important into insights in some cases. In other cases, it has mainly given us a glimpse of what may be possible in physiological settings. The challenge of the next few years will be to separate what is only theoretically possible from what actually happens in cells in which the receptor is naturally expressed.

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Serotonin Receptors: Clinical Implications

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GLENNON, R. A. *Serotonin receptors: Clinical implications*. NEUROSCI BIOBEHAV REV 14(1) 35–47, 1990.—Over the past decade, a variety of serotonin (5-hydroxytryptamine, 5-HT) receptor/binding sites have been identified. These include 5-HT_1, 5-HT_2, and 5-HT_3 sites. The 5-HT_1 sites have been further divided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1E} sites. It would be of interest to identify those pharmacological effects that are specifically mediated by a particular population of 5-HT sites and, indeed, attempts have been made to do this almost since the initial discovery of multiple populations of sites. Unfortunately, much of the early work made use of serotonergic agents that are now known to be somewhat less selective than originally suspected. Nevertheless, there is ample information in the literature suggesting that site-selective serotonergic agents may ultimately lead (and, in some cases, has already led) to the development of therapeutically-useful agents. The present review examines the pharmacological effects that are thought to be related to the individual types of 5-HT sites and provides some clinical implications for agents that act at these sites.

Serotonin 5-HT_1 5-HT_2 5-HT_3 Serotonin receptors

THE pioneering work on radioligand-binding techniques during the 1970s resulted in an explosive growth in neurotransmitter research in the 1980s. Serotonin (5-hydroxytryptamine, 5-HT) was one of the neurotransmitters that essentially underwent a re-birth during this time. With the discovery in late 1979 that there exists more than one type of central 5-HT binding site (141), publications on this neurotransmitter have appeared at an unprecedented rate. Subsequent studies attempted to identify site-selective ligands, and with these novel agents came attempts to delineate the functional significance of the newly discovered 5-HT_1 and 5-HT_2 sites. Since that time, several populations of 5-HT binding sites have been reported: 5-HT_1, 5-HT_2, 5-HT_3, and, most recently, 5-HT_4. The 5-HT_1 sites have been further subclassified as: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1E}. Unfortunately, selective ligands have not yet been developed for some of these sites and/or the sites are too newly discovered to have had their pharmacology investigated in any detail. Several recent reviews on the discovery and characterization of 5-HT binding sites, and on the selectivity of agents that interact with these sites, have been published (28, 52, 63, 67, 134, 139, 150, 152). Some of these sites, such as the 5-HT_{1E} sites (165) and the 5-HT_4 sites (45) are too new to have been described in any of the available reviews.

The purpose of this present review is to explore aspects of the physiological relevance and, where possible, the potential clinical implications of several of the better studied 5-HT binding sites. Shortly after the initial characterization of 5-HT_1 and 5-HT_2 binding sites came suggestions as to their functional relevance. The continued discovery of new populations of sites, and in particular, of multiple populations of 5-HT_1 binding sites, has made it difficult to ascribe roles for each of these sites with any degree of confidence. For example, early studies attempted to describe the potential functional significance of those sites labeled by [^{3}H]5-HT (i.e., 5-HT_1 sites); today, at least five different populations of 5-HT sites can be labeled by this radioligand. In addition, subsequent speculation regarding the clinical significance of 5-HT_{1B} sites has been dampened by reports that 5-HT_{1D} sites may not be present in human brain. As a consequence, some of the proposed roles for 5-HT sites are still controversial. Adding to the confusion is the finding that selectivity can be a temporal phenomenon; agents selective today may not be selective tomorrow. That is, certain agents, once thought to be selective for a particular population of 5-HT binding sites, may now be considered (a) nonselective, (b) more selective for another (more recently discovered) 5-HT binding site, or (c) more selective for (or at least equi-effective in binding at) another neurotransmitter binding site. Conversely, certain nonserotonergic agents bind with high affinity at 5-HT sites. Two of the most important examples are the alpha-adrenergic agent WB-4101 and beta-adrenergic antagonists such as pindolol and propranolol; under the appropriate conditions, [^{3}H]WB-4101 can label 5-HT_{1A} sites, and pindolol and propranolol are routinely employed now as 5-HT_{1A} antagonists. There is also evidence that some agents may act as agonists at one 5-HT site and as antagonists at another population of 5-HT sites (68). As a consequence, there is a need to reinterpret, or at least carefully interpret, the results of some of the older studies. Nevertheless, there is still a sufficient amount of information available from which to implicate a role for 5-HT in a variety of physiological processes and pharmacological responses. Where evidence implicating the involvement of a particular site is sparse, a liberal view is presented with the understanding that additional research may be required. This review covers the literature up to the Spring of 1989; however, greater emphasis is placed on the more recent literature. An attempt has been made to be fairly

comprehensive; nevertheless, the main goal is to describe some of the promising areas of study and to identify the names of key investigators for those who wish to do additional reading on these topics.

Specific topics to be covered in this review include (a) feeding behavior, (b) thermoregulation, (c) sexual behavior, (d) cardiovascular/hypotensive activity, (e) sleep, (f) antipsychotic activity, (g) anxiolytic/antiaggressive activity, (h) depression, and (i) hallucinogenic activity. Some closing comments are made on the possible involvement of 5-HT in neurodegenerative disorders and on the potential application of 5-HT_3 antagonists in the treatment of migraine and chemotherapy-induced nausea and vomiting. It should be kept in mind that much of the pharmacology of 5-HT systems is only as good as the selectivity of the agents used to explore this pharmacology. As discussed above, the selectivity of these agents is subject to change; consequently, the subsequent discussions may eventually be subject to corresponding changes. For purpose of convenience, the relative selectivity of many of the agents used in the following discussion is listed in Table 1.

FEEDING BEHAVIOR

Feeding is subject to numerous (redundant) inhibitory influences (156). Stimulation of inhibitory 5-HT autoreceptors by 5-HT_{1A} agonists produces hyperphagia in free-feeding rats (43, 91, 92). Montgomery and co-workers (122) have suggested that 8-OH DPAT-induced hyperphagia may be secondary to the elicitation of chewing behavior. However, Dourish *et al.* (42) have argued against a nonspecific gnawing mechanism on the basis that partially satiated rats are exceptionally sensitive to 8-OH DPAT and that this agent is more effective in partially satiated rats than in nondeprived rats. Furthermore, 8-OH DPAT attenuates fenfluramine-induced anorexia (42), which is thought to depend on increased serotonergic neurotransmission. Cooper (25) has suggested that 8-OH DPAT inhibits a serotonergic mechanism that mediates satiation in nondeprived animals (i.e., 8-OH DPAT enhances food consumption by virtue of a de-satiation effect). Because 8-OH DPAT can antagonize fenfluramine-induced anorexia, fenfluramine presumably acting via a serotonergic satiation mechanism, it has been further suggested that 8-OH DPAT and fenfluramine produce opposite effects on the same satiation system (25). The 5-HT_{1A} agonists, or mixed agonist/antagonists, gepirone, ipsapirone, and buspirone failed to inhibit 8-OH DPAT-induced feeding; rather, consistent with an agonist mechanism, these agents dose-dependently increased feeding when given alone (44,59). Selective pre- versus postsynaptic 5-HT_{1A} agonists and antagonists may ultimately be of utility in treating obesity and eating disorders; however, to date, selective agents of this nature have not been reported.

Stimulation of postsynaptic 5-HT_{1B} receptors seems to account for the hypophagic effect produced by RU 24969 (98). Certain arylpiperazines such as mCPP produce anorexia in rats (151). However, although mCPP and TFMPP also bind at 5-HT_{1B} sites, there is evidence that their hypophagic effects may require a 5-HT_{1B} and 5-HT_{1C} mechanism. One explanation provided by Kennett and Curzon (98) is that mCPP and TFMPP act by directly stimulating 5-HT_{1C} receptors which lead, in turn, to stimulation of 5-HT_{1B} receptors. This hypothesis implies that mCPP and TFMPP have little direct stimulatory effect on 5-HT_{1B} receptors that mediate hypophagia. Involvement of 5-HT_2 and 5-HT_3 receptors in the hypophagic effects of mCPP seem unlikely (98); however, the 5-HT_3 antagonist ICS 205,930 potentiated the effect of mCPP and suggests an interaction between 5-HT_{1C} and 5-HT_3 receptors in the control of food intake (98). Mianserin, mesulergine, metergoline, and 1-NP, but not cyproheptadine, all of which bind at 5-HT_{1C} and 5-HT_2 sites, also attenuate the effect of mCPP (98). Given alone, mianserin, 1-NP, and cyproheptadine increase food consumption by normally fed rats, but not by food-deprived rats, presumably via a 5-HT_{1C} antagonist mechanism (98). By themselves, the 5-HT_2-selective antagonists ritanserin, ketanserin, and LY 53857 do not enhance food consumption (115,156). Although neither ritanserin nor ketanserin antagonize the hypophagic effect of mCPP (98), ketanserin can reverse the anorexic effect of fenfluramine (84). The putative 5-HT_2 agonist DOI, and it may be parenthetically noted that DOI and related agents also bind at 5-HT_{1C} sites (166,167), inhibits feeding in a dose-related manner; this effect can be antagonized by ketanserin, LY 53857, and 1-NP (156). 1-NP, but not ketanserin or LY 53857, reduced food consumption when given alone and the effect of 1-NP was attributed to activation of a 5-HT_{1B} mechanism (156). Interestingly, the peripherally acting 5-HT_2 antagonist xylamidine is capable of attenuating the anorexic effects of 5-HT but fails to block the effect of DOI (156). Although these results suggest that DOI may produce its effects via a central 5-HT_2 mechanism, the effect of xylamidine at 5-HT_{1C} receptors is unknown. Agents structurally related to DOI, such as DOM and DOB, also reduce food intake in dogs (176). Because DOI, DOM and DOB are hallucinogenic agents, the possibility exists that these agents reduce food intake due to their hallucinogenic or general disruptive actions. Though this issue has yet to be resolved, Schechter and Simansky (156) have demonstrated that a several-fold higher dose of DOI was necessary in to reduce water intake relative to the dose necessary to reduce food consumption. Nevertheless, due to the hallucinogenic actions of these DOI-related agents, it is highly unlikely that they will find any clinical utility as appetite-suppressants. There is, however, an interesting possibility that should not be overlooked. Hallucinogenic activity, as will be discussed below, may be related either to 5-HT_2 and/or 5-HT_{1C} agonism; if this activity is due solely to one of these two mechanisms and anorectic activity is related to the other, it is theoretically feasible to obtain appetite-suppressants that are devoid of hallucinogenic properties.

Finally, inconsistencies have been reported in the literature with regard to the effect of various agents on food consumption. It is now recognized that numerous experimental factors can influence the results of such studies. Recently, it was reported that housing and pretest handling of the animals also need to be considered (26).

THERMOREGULATION

It should perhaps be mentioned at the outset that there is now conclusive evidence (a) that 5-HT plays a role in thermoregulation, (b) that there exist both sex and species differences with regard to the effects of various agents/treatments, and (c) that dose and environmental factors are critical variables. The 5-HT_{1A} agonist 8-OH DPAT produces hypothermia in rodents (73, 79, 87). This effect can be antagonized by the 5-HT_{1A} antagonist (−)pindolol, the 5-HT_{1A}/5-HT_2 antagonist spiperone, and the 5-HT_1/5-HT_2 antagonists pizotyline and methiothepin, but not by mianserin, or the 5-HT_2 antagonists ketanserin, pirenperone, or ritanserin (76,79); metergoline enhances the hypothermic effect of 8-OH DPAT (76). (−)Propranolol can block the effect of 8-OH DPAT in rats but not in mice (76). There are sex-related differences in responsiveness of 5-HT_{1A} receptors that mediate hypothermia; female rats exhibit a much more pronounced decrease in body temperature to 8-OH DPAT than do male rats (16,79). The second generation anxiolytic agents buspirone, gepirone, and ipsapirone also produce hypothermia in rats and this effect can be antagonized by (−)pindolol and spiperone, but not

TABLE 1

AGENTS COMMONLY EMPLOYED TO INVESTIGATE SEROTONERGIC MECHANISMS*

Agent	Relative Selectivity
BRL 43694	5-HT_3 antagonist
Buspirone	A-SGA† with affinity for 5-HT_{1A} sites
Cinanserin	5-HT_2 antagonist
Cyproheptadine	5-HT_2 antagonist; antihistamine
DOB	5-HT_2 agonist (also binds at 5-HT_{1C} sites)
DOI	5-HT_2 agonist (also binds at 5-HT_{1C} sites)
DOM	5-HT_2 agonist (also binds at 5-HT_{1C} sites)
Fenfluramine	5-HT releasing agent
Gepirone	A-SGA with affinity for 5-HT_{1A} sites
GR 38032(F)	5-HT_3 antagonist
ICS 205,930	5-HT_3 antagonist
Iodocyanopindolol	5-HT_{1B} ligand; 5-HT_{1B} antagonist?
Ipsapirone	A-SGA with affinity for 5-HT_{1A} sites
Ketanserin	5-HT_2 antagonist with affinity for 5-HT_{1C}, DA and other neurotransmitter sites
LSD	Binds at 5-HT_1 and 5-HT_2 sites in a nonselective manner; binds at dopamine sites. Does not bind at 5-HT_3 sites.
LY 53857	5-HT_2 antagonist lacking adrenergic antagonist activity associated with ketanserin
mCPP	Considered a 5-HT_{1B}/5-HT_{1C} agonist; also binds at 5-HT_3 sites
MDL 72222	5-HT_3 antagonist
Mesulergine	Binds with high affinity at 5-HT_{1C}/5-HT_2 sites
Metergoline	Relatively nonselective 5-HT_1/5-HT_2 antagonist
Mianserin	5-HT antagonist with greatest affinity at 5-HT_2 and 5-HT_{1C} sites
PAPP (LY 165163)	5-HT_{1A} agonist
Pindolol	Beta-adrenergic antagonist; 5-HT_{1A} antagonist
Pirenperone	5-HT_2 antagonist with affinity for 5-HT_{1C} and other neurotransmitter sites
Pizotyline (BC 105)	5-HT_2 antagonist; binds at certain 5-HT_1 sites.
Propranolol	Beta-adrenergic antagonist; 5-HT_{1A} antagonist
Ritanserin	5-HT_2 antagonist with affinity for 5-HT_{1C} and other neurotransmitter sites. Considered more selective than ketanserin and pirenperone.
Quipazine	5-HT_{1B}/5-HT_2/5-HT_3 ligand
RU-24969	Considered a 5-HT_{1B} agonist; displays considerable affinity for 5-HT_{1A} sites
Spiperone	5-HT_{1A}/5-HT_2 and dopamine antagonist
TFMPP	Considered a 5-HT_{1B} agonist; displays affinity for other 5-HT (particularly 5-HT_{1C}) sites
Xylamidine	5-HT_2 antagonist; does not readily penetrate the blood-brain barrier
1-NP: (1-(1-Naphthyl)piperazine)	Binds at 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 sites
2-Methyl 5-HT	5-HT_3 agonist
5-CT; 5-CAT: (5-Carboxamidotryptamine)	Binds at all (except 5-HT_{1E}) 5-HT_1 sites with high affinity. Di-n-propyl analog more 5-HT_{1A}-selective.
5-OMe DMT	Nonselective 5-HT agonist
8-OH DPAT	Selective 5-HT_{1A} agonist

*The selectivity of these agents is based primarily on radioligand binding data, but, as listed, reflects their commonly accepted application. For actual binding data, see (67, 139, 152). For structures of these agents, see appendix to (152) and (150).

†A-SGA = Arylpiperazine second-generation anxiolytic.

by ketanserin (79). Interestingly, in mice, ipsapirone does not produce hypothermia and can, in fact, block the hypothermic response produced by 8-OH DPAT (76). However, Maj and co-workers have reported that ipsapirone partly inhibits the effect of 8-OH DPAT in rats, but not in mice, and that at high doses ipsapirone produces hypothermia in both species (111). The

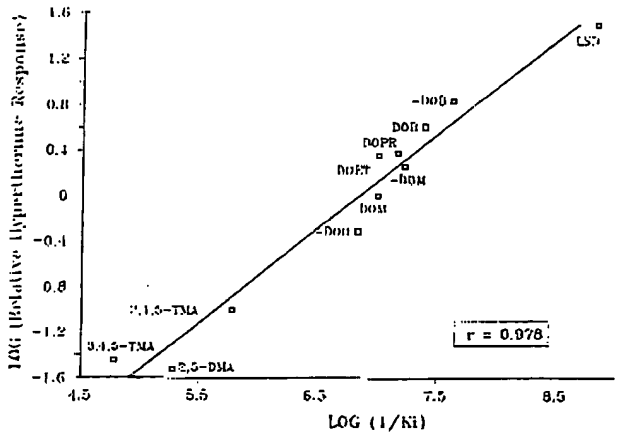

FIG. 1. Correlation between the relative hyperthermic potencies of (+)LSD and ten phenylalkylamine derivatives and their affinities for [^{3}H]ketanserin-labeled 5-HT$_2$ binding sites. Hyperthermia potency data are from Aldous and co-workers (3); 5-HT$_2$ binding data are from Titeler and collaborators (167).

putative 5-HT$_{1A}$ agonist PAPP (LY 165163) also produces a significant and dose-dependent hypothermia in rats (90). There is some dispute as to whether these 5-HT$_{1A}$-mediated hypothermic effects arise from a presynaptic (76) or postsynaptic (90) mechanism.

Certain arylpiperazines, such as mCPP, produce hyperthermia in rats (111) and in humans (124). Martin and co-workers (114) have found that arylpiperazines with high affinity for 5-HT$_{1A}$ sites produce hypothermia whereas arylpiperazines with high affinity for 5-HT$_{1B}$ sites produce hyperthermia. Further, the hyperthermic effects of these agents are antagonized by metergoline whereas the hypothermic effects are antagonized by (−)propranolol (114). Pirenperone antagonized mCPP- and quipazine-induced hyperthermia leading Pawlowski to conclude that a 5-HT$_2$ mechanism might be involved (138).

5-HT$_2$ agonists appear to produce hyperthermia in rodents. The hyperthermic effect of MK-212 has been attributed to a 5-HT$_2$ agonist mechanism on the basis that it can be antagonized by mianserin, pizotyline, spiperone, ketanserin, and pirenperone, but not by (−)pindolol (79). Furthermore, the effect is presumably centrally mediated in that it is not antagonized by xylamadine (79). The putative phenylalkylamine 5-HT$_2$ agonists such as DOB and DOM have long been known to produce hyperthermia in animals [e.g., see references (3,64) for a brief review]. Furthermore, the actions are stereoselective in that, where examined, the R(−)-isomers are approximately an order of magnitude more potent than their S(+)-enantiomers (3). (+)LSD also produces a significant hyperthermic response in animals (3); however, its mechanism of action in this regard has not yet been determined. We have recently published 5-HT$_2$ binding data on 10 such phenylalkylamines, in comparison with (+)LSD, using both tritiated ketanserin and tritiated DOB as radioligands. There is a significant correlation between the affinities of these 11 agents at 5-HT$_2$ sites and their relative hyperthermic potencies [employing the data of Aldous and co-workers (3)] in rabbits (r = .978 and .944, respectively) (Glennon, unpublished finding; see Fig. 1). It might be noted that a significant correlation (r = .921) also exists between the relative hyperthermic potencies of these same agents and their affinities for 5-HT$_{1C}$ sites (Glennon, unpublished findings). The nonselective 5-HT agonist 5-OMe DMT produces hypothermia at low doses and hyperthermia at higher doses; this is thought to be due to its actions at 5-HT$_{1A}$ and 5-HT$_2$ receptors, respectively (79).

SEXUAL BEHAVIOR

In general, 5-HT agonists have an inhibitory effect on the mediation of sexual behavior in male rats (1). Thus it was surprising to find that 8-OH DPAT seemingly produces a paradoxical facilatory effect (1,2). Because some dopamine agonists (such as apomorphine and 5-OH DPAT) and mixed 5-HT/dopamine agonists (such as lisuride) produce a similar effect, Ahlenius and Larsson (1) examined a potential dopaminergic role for 8-OH DPAT; they found that the dopamine antagonist haloperidol antagonizes the effect of apomorphine and 5-OH DPAT, but not that of 8-OH DPAT. Further, (−)alprenolol and (−)pindolol partly antagonize the effect of 8-OH DPAT, but seem to have an effect of their own. 8-OH DPAT was not antagonized by methiothepin, metergoline, or pirenperone, but, 8-OH DPAT was able to antagonize the inhibitory effect of the 5-HT precursor 5-hydroxytryptophan (2). It was suggested that 8-OH DPAT may act as an antagonist at postsynaptic 5-HT$_{1A}$ sites, and/or as an agonist at presynaptic 5-HT$_{1A}$ sites (1). Olivier and co-workers (132) reported that 8-OH DPAT, buspirone and ipsapirone produce qualitatively similar effects in male rats but that the effects are dependent upon the behavioral status and sexual experience of the animal. On the other hand, 8-OH DPAT has an inhibitory effect on sexual activity in the male mouse (164). 8-OH DPAT also seems to have an inhibitory role on female rat receptivity, and sensitivity to 5-HT$_{1A}$ agonists may be high in those stages of the cycle when sexual behavior is inhibited (32). Estrogens have also been found to enhance electrophysiological responses to 5-carboxamidotryptamine in female rat hippocampus (21) suggesting that serotonergic agents may act indirectly by modifying the effect of hormones. BAY R 1531, an indolic compound related in structure to 8-OH DPAT, produces effects on male rat sexual behavior similar to those of 8-OH DPAT (62). Sexual activity in animals may be related to aggression (130); if this is the case, it is not surprising to find that 5-HT$_1$ mechanisms are also implicated in aggressive behavior.

Several groups have now reported that 5-HT$_{1B}$ receptors may be involved in sexual behavior (117,132) and 5-HT$_{1B}$ agonists produce different effects on male and female rats. mCPP, TFMPP and RU 24969 facilitate sexual behavior in female rats (117); and inhibit behavior in male rats (117,132). 5-HT$_2$ receptors may have an inhibitory role in male rat sexual behavior; DOI suppresses activity whereas LY 53857 amplifies sexual behavior (50). LY 237733 is 1000 times more potent than LY 53857 in this regard (50). In estrogen-primed ovariectomized female rats, the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ agents 8-OH DPAT, mCPP, and mesulergine, respectively, inhibited sexual activity, whereas DOI was stimulatory (88). Mendelson and Gorzalka (118) have suggested that 5-HT$_3$ receptors may have an inhibitory role on lordosis in the female rat. However, the 5-HT$_3$ antagonist GR 38032, but not MDL 72222, was found to stimulate female sexual behavior (88).

To date, there is no information on the effect of 5-HT site-selective agents on human sexual activity.

CARDIOVASCULAR/HYPOTENSIVE EFFECTS

5-HT exerts complex cardiovascular effects that depend upon the species of animal, the vascular bed under study, the dose of drug, and the experimental conditions employed in the investigation (154). Effects can result from the direct or indirect actions of 5-HT. Gothert (74) and Saxena (153) have reviewed the involvement of 5-HT receptors in the circulatory system and in mammalian heart, respectively. For general discussions of the cardiovascular effects of serotonergic agents, see Vanhoutte (173), Frohlich and van Zwieten (53), Breckenridge *et al.* (10), and Saxena *et al.* (155).

The major peripheral actions of 5-HT appear to be on the blood vessel wall where it can cause either contraction (vasoconstriction) or relaxation (vasodilation) of vascular smooth muscle (172). Vasoconstriction may involve a 5-HT_2 mechanism, although in certain blood vessels (e.g., coronary and cerebral arteries), this effect may be 5-HT_1-mediated (172). Some vascular effects involve non-5-HT_1/non-5-HT_2 5-HT receptors. Serotonin is also released in significant quantities during platelet aggregation. As such, it further enhances platelet aggregation (most likely via 5-HT_2 receptors), and causes vasoconstriction both by direct action and by amplification of the contractile response to, for example, norepinephrine (172,174).

Serotonin can lower blood pressure by several different mechanisms. For example, via inhibition of central vasomotor loci, which involves 5-HT_1-like (5-HT_{1A}?) receptors, and by inhibition of release of norepinephrine from postganglionic sympathetic neurons, which may also involve 5-HT_1-like receptors (154,174). 5-HT can cause indirect relaxation of some vascular smooth muscle. With regard to blood pressure, 5-HT elicits a tri-phasic response consisting of an initial transient hypotensive response, a pressor phase, and a longer lasting depressor phase. These effects appear to be mediated by 5-HT_3-, 5-HT_2-, and 5-HT_1-like receptors, respectively (154,174). 5-HT can also release vasodilator substances from vascular endothelium (174).

Administered directly into the brain, and depending upon location of administration, 5-HT produces inhibitory and excitatory effects on sympathetic nerve discharge (SND) and blood pressure. McCall and Harris (107,108) examined the effect of various selective serotonergic agents on blood pressure (mean arterial pressure), heart rate, and SND using cats as subjects. They found that 8-OH DPAT and PAPP decreased all three measures suggesting involvement of a central 5-HT_{1A} mechanism. Mir and Fozard (119) also reported that the hypotension and bradycardia produced by 8-OH DPAT in cats and in rats reflects primarily a decrease in central sympathetic tone. Ipsapirone and flesinoxan (DU 29373), with appreciable affinity and selectivity for 5-HT_{1A} sites, also produce a central hypotensive action (119). TFMPP, mCPP, and 2-MPP produced variable effects on SND, and minimal effects on arterial pressure and heart rate. DOI produced a massive increase in SND, an increase in blood pressure, and essentially no change in heart rate; MK-212 produced similar, but smaller, effects (107,108). A 5-HT_2 mechanism was implicated for DOI on the basis that its effects could be antagonized by ketanserin and LY 53857. The increase in blood pressure in the presence of minimal changes in heart rate suggested an additional peripheral vasoconstrictor action for DOI. Thus, it is found that one subset of 5-HT receptors (5-HT_{1A}) can inhibit SND, whereas a second set (5-HT_2) increases SND.

Certain 5-HT_2 antagonists, most notably ketanserin, produce a hypotensive effect and have been demonstrated to lower blood pressure in animals including humans. There has been considerable controversy as to whether the hypotensive effect produced by ketanserin involves a 5-HT_2 and/or alpha-adrenergic mechanism. Amongst some of the latest clinical reports on the subject are one by Naslund *et al.* (126) suggesting that the adrenergic component makes a minimal contribution, and another by Hosie *et al.* (89) suggesting that the acute antihypertensive effects of ketanserin are not attributable solely to a 5-HT_2 mechanism. The latter study also demonstrates that ritanserin does not produce ketanserin-like hypotensive effects. Saxena *et al.* (154) have commented that ketanserin may lower arterial blood pressure by four different mechanisms: blockade of 5-HT_2-mediated vasoconstrictor effects, alpha-1 adrenergic blockade, inhibition of central vasomotor loci, and direct vasodilation. van Zwieten and co-workers (175) have reported that the central hypotensive effects of ketanserin in cats may involve neither a serotonergic nor an adrenergic mechanism. Regardless of its mechanism of action, agents such as ketanserin may be of benefit in the treatment of hypertension, peripheral vascular disease, coronary and cerebral vasospasm, Raynauds phenomenon, carcinoid syndrome, and migraine (174).

SLEEP

There is considerable evidence that 5-HT is involved in the regulation of the sleep-waking cycle [e.g., see (101,109) for general reviews]. Much less is known, however, concerning the effects of site-selective serotonergic agents. The 5-HT_{1A} agonist 8-OH DPAT dose-dependently delays the first appearance of REM sleep in rats, and low doses of 8-OH DPAT decrease the number of wakenings whereas higher doses increase wakefulness (38). TFMPP produces a dose-related short-lasting decrease in REM sleep in rats by increasing REM latency (137). Quipazine suppresses both REM and NREM sleep in rats (51). The 5-HT_2 antagonist ritanserin increases NREM sleep and depresses REM sleep in rats and humans, whereas the 5-HT_2 antagonists ICI 169,369 and ICI 170,809 suppress REM sleep but have no significant effect on NREM sleep (136). Another 5-HT_2 antagonist LY 53857 also suppresses REM sleep, but increases NREM sleep in a nondose-dependent fashion. This has led to the conclusion that 5-HT_2 antagonists consistently suppress REM sleep but have an inconsistent effect on NREM sleep (136). Conversely, the putative 5-HT_2 agonist DOI increases REM sleep (135).

The clinical utility of agents that influence sleep and wakefulness is readily apparent. However, according to Koella's "general theory of vigilance" (101), 5-HT may be involved with aspects of vigilance other than sleep, and in a more integrative fashion may influence cognition, learning, perception, memory, decision making, mood and behavior. Although more will be mentioned about mood and behavior in subsequent sections, relatively little has been published on the effects of site-selective serotonergic agents on these other processes.

ANTIPSYCHOTIC ACTIVITY

Members of every major class of neuroleptic agents bind with significant (usually low nanomolar) affinity at 5-HT_2 sites [see reference (63, 105, 134) for brief discussions]. At this time, there is no compelling reason to believe that agents that bind solely at 5-HT_2 sites possess neuroleptic activity; however, at least in animal studies, many neuroleptic agents act as 5-HT_2 antagonists [e.g., (63, 105, 133, 134)]. Previously, there have been several reports suggesting a decrease in 5-HT_2 binding sites in the post-mortem brain of schizophrenic patients; Mita and co-workers (120) have recently reported similar findings in two groups of patients who were either being treated with neuroleptic agents, or where neuroleptic treatment had been discontinued several months prior to death. They suggest that the decrease in the number of 5-HT_2 sites in prefrontal cortex may be related to the disease process, per se (120). These findings, coupled with the high affinity of neuroleptic agents for 5-HT_2 sites, raise intriguing questions regarding the role of 5-HT_2 sites in schizophrenia. However, a recent analysis of 17 neuroleptic agents using normal human brain frontal cortex homogenates revealed that all possess low affinity for 5-HT_{1A} sites (Ki values ranging from 230 to 40,000 nM) and high affinity (Ki values ranging from 0.38 to 130 nM, with the exception of molindone, Ki = 5000 nM) for 5-HT_2 sites (177). Because there is no correlation between 5-HT_2 affinity and average daily dose for treating schizophrenia ($r = .097$), a 5-HT_2 interaction by itself does not seem to explain neuroleptic potency.

Several atypical neuroleptic agents bind with high affinity both at dopamine sites and 5-HT_2 sites. Examples of newer agents with a significant affinity for 5-HT_2 sites and with clinical potential

include: amperozide (80,116), befiperide (171), tefludazine (163), setoperone (8), risperidone (94), and ritanserin (6). Furthermore, the 5-HT_2 antagonist ritanserin, which seems to be, at best, a weak dopamine antagonist (6), demonstrated clinical usefulness in the treatment of chronic schizophrenia when compared to haloperidol (58). Taken together, these results suggest further research in this area is warranted.

The 5-HT_3 antagonist GR 38032F has been demonstrated to reduce the behavioral consequences of chronic infusion of dopamine into the mesolimbic nucleus accumbens (12). This agent is also capable of antagonizing the behavioral effect of a neurokinin receptor agonist whose actions are accompanied by increases in dopamine metabolism in the mesolimbic and mesocortical regions of the forebrain (81). Such results have led to speculation that there are 5-HT_3-mediated systems that serve to amplify activating influences on mesolimbic dopaminergic neurons and that 5-HT_3 antagonists may have some potential neuroleptic activity (81).

ANXIOLYTIC/ANTIAGGRESSIVE ACTIVITY

5-HT_{1A}-Related

An exciting finding is that certain serotonergic agents may constitute a novel mechanistic class of antianxiety agents. Although benzodiazepine anxiolytic agents are known to affect serotonergic systems [e.g., (47)], their primary mechanism of action is believed to involve interaction at central benzodiazepine receptors. Nevertheless, there is a considerable literature implicating a role for serotonin in anxiety; for a review, see Gardner (54,55). The azaspirodecanone buspirone was initially developed as a potential antipsychotic agent, and although it appeared to be only of limited value in clinical studies, subsequent animal studies suggested that it might possess antianxiety activity. Indeed, this was shown to be the case: see Dourish (40) for a recent review of some early clinical trials. Shortly thereafter, two related derivatives, ipsapirone (previously referred to as TVX Q 7821 and isapirone) and gepirone, were also demonstrated to display anxiolytic activity. Together, these three agents represent some of the best-studied examples of nonbenzodiazepine arylpiperazine second-generation anxiolytics (A-SGAs) and literally hundreds of papers and abstracts have appeared on these and related agents in the last few years. For recent reviews of this topic, see: Gardner (56), Traber and Glaser (168), and Young and Glennon (180).

Early studies with buspirone were not altogether convincing in implicating a role for serotonin; likewise, buspirone was not significantly active in some animal models normally predictive of anxiolytic activity. Indeed, all three A-SGAs give what appear to be conflicting results in various animal models for anxiolytic activity, or, are inactive in certain animal models [see (40, 56, 168, 180) for reviews]. Although most investigations now implicate a role for 5-HT in the mechanism of action of these agents, there is also evidence that the activity in some animal models may be related to a dopaminergic component [e.g., (143)]. Although a complete discussion of such studies is well beyond the scope of this review, it might be noted that in at least one animal model (the elevated plus maze) buspirone, ipsapirone, and 8-OH DPAT appear to produce anxiogenic effects (29,123) and that the anxiogenic effect of 8-OH DPAT can be antagonized by ipsapirone (30,31). In fact, Broekkamp and Jenck have commented that no single animal model is universally applicable for identifying all types of anxiolytic agents (13). Perhaps three of the most important reasons for this are (a) anxiety in humans may not be a homogeneous disorder and exact parallels may not exist in animal models, (b) most animal models were developed using benzodiazepine anxiolytic agents and may be useful for identifying new benzodiazepine anxiolytics, but not anxiolytics with a somewhat different profile (or mechanism) of action, and/or (c) because buspirone, gepirone, and ipsapirone all give rise to a common metabolite, 1-(2-pyrimidinyl)piperazine (1-PP or 1-PmP), that may be an active metabolite responsible for the anxiolytic effects of these agents (15), but that is, at the same time, a metabolite that for some reason is not identified by certain animal models. (This latter possibility now seems unlikely and will be further discussed below.) Trazodone, another agent reported to produce anxiolytic activity, is also metabolized to a structurally-related arylpiperazine, mCPP.

Buspirone, gepirone, and ipsapirone do not bind with significant affinity at benzodiazepine-binding sites, but do bind at 5-HT_1 sites (61, 140, 169). They also display a significant affinity for 5-HT_{1A} sites relative to other 5-HT populations (46, 75, 140, 166). Other investigators have since replicated these findings. Ipsapirone itself has been tritiated and used as a radioligand and appears to label 5-HT_{1A} sites (39,169). The arylpiperazine mCPP also binds at 5-HT_{1A} sites, but is neither selective nor of high affinity (Ki = ca 150 nM); 1-PP possesses an even lower affinity at 5-HT_{1A} sites (e.g., 5-HT_{1A} Ki = 1520 nM, relative to buspirone, Ki = 15 nM) (70).

A considerable amount of drug discrimination work has been conducted in order to determine the mechanism of action and similarity of effects of the A-SGAs, and whether or not the A-SGAs and benzodiazepines, such as diazepam, produce similar stimulus effects. Stimulus generalization occurs with the A-SGAs buspirone, gepirone, and ipsapirone in rats trained to discriminate the 5-HT_{1A} agonist 8-OH DPAT from saline; see Young and Glennon (180) and Glennon (66) for reviews. Likewise, stimulus generalization occurs with 8-OH DPAT in animals trained to discriminate either buspirone (113), ipsapirone (70,162) or gepirone (78) from saline. Neither the A-SGAs nor 8-OH DPAT are recognized by animals trained to discriminate diazepam from saline, nor is diazepam recognized by animals trained to discriminate either 8-OH DPAT or ipsapirone (180). Other benzodiazepines also fail to produce stimulus effects similar to those produced by 8-OH DPAT or the A-SGAs [e.g., (113,180)]. The diazepam stimulus, but not the 8-OH DPAT stimulus, can be antagonized by the benzodiazepine antagonist flumazenil (Ro 15,1788) (180), whereas the 8-OH DPAT stimulus can be antagonized by (−)pindolol (170), an agent known to act as a 5-HT_{1A} antagonist. Apparently, then, the A-SGAs and 8-OH DPAT produce similar stimulus effects, but stimulus effects that differ from those produced by typical benzodiazepine anxiolytics. These results support the notion that the A-SGAs most likely produce their effects by a mechanism that differs from that involved for the benzodiazepines, and one that possibly involves 5-HT_{1A} mediation. The 8-OH DPAT stimulus fails to generalize to either 1-PP or mCPP; indeed, in all studies using animals trained to agents thought to produce their stimulus effects via either a selective or nonselective 5-HT_{1A} mechanism, no stimulus generalization is observed with 1-PP [e.g., (161,180)]. 1-PP is also inactive in most animal models of anxiety (56, 180, 181). These data make it seem unlikely that 1-PP is responsible for the stimulus effects produced by the A-SGAs. Indeed, it has been reported that mCPP produces an anxiogenic response in humans (20); its mechanism of action in this regard is unknown. Because there is evidence that 1-PP can interact at alpha-2 adrenergic sites (19), 1-PP may also be an anxiogenic agent. Although 1-PP may not be responsible for the stimulus effects of the A-SGAs, it should not be considered pharmacologically inactive and may indeed account for certain effects produced by these agents [e.g., (60)].

Another problem with the A-SGAs (as well as with 8-OH DPAT), is that they behave as (5-HT_{1A}) agonists in some pharmacological assays and as antagonists in others. Many of the effects of the A-SGAs might be better understood if they were acting as partial agonists, or if they were acting differently at pre-

vs. postsynaptic sites; indeed, there is some evidence for this (41). There are also suggestions that the anxiolytic effects of buspirone do not involve a 5-HT_{1A} mechanism [e.g., (34)], and other potential mechanisms should not be completely eliminated from consideration. Nevertheless, a 5-HT_{1A} mechanism seems most likely at this time, and Dourish has recently reviewed the literature suggesting that these agents produce their anxiolytic effects via either a presynaptic agonist mechanism or via a postsynaptic antagonist mechanism involving 5-HT_{1A} receptors (40).

In summary then, it appears that (a) the A-SGAs are effective anxiolytic agents in humans although they are not consistently active in all animal models of anxiety, (b) they bind with significant affinity and selectivity at 5-HT_{1A} sites, (c) they produce stimulus effects common to one another but distinct from those produced by benzodiazepines or other nonbenzodiazepine anxiolytic agents, and (d) it is unlikely that certain metabolites (e.g., 1-PP, mCPP) are primarily responsible for anxiolytic activity.

5-HT_{1B}-Related

The A-SGAs display relatively low affinity for 5-HT_{1B} sites; nevertheless, mCPP possesses a somewhat higher affinity for 5-HT_{1B} sites than it does for 5-HT_{1A} sites. This led to some early speculation that 5-HT_{1B} sites might be involved in anxiety. Other agents with some (though, at best, marginal) selectivity for 5-HT_{1B} sites have also been evaluated in models of anxiolytic activity (e.g., TFMPP, RU-24969). To date there is little evidence that 5-HT_{1B} sites are involved in anxiolytic activity (56,180).

5-HT_2-Related

Certain classical 5-HT antagonists, most of which are now known to be fairly selective 5-HT_2 antagonists, were known to be active in animal models of conflict [see (180) for a brief review]. Early on, the antidepressant trazodone was demonstrated to be both a serotonin agonist and antagonist, and it was speculated that the agonist effect of trazodone might be related to formation of an active metabolite (112). Trazodone antagonizes effects that are now thought to be 5-HT_2-mediated [e.g., head-twitch (22)]. Subsequently, trazodone was shown to bind with significant affinity at 5-HT_2 sites and tetrahydrotrazodone antagonizes the discriminative stimulus effects of the putative 5-HT_2 agonist DOM (69). Eventually, trazodone was found to be of benefit in the treatment of anxiety (4). Since then, other 5-HT_2 antagonists have been shown to possess anxiolytic properties. Of these, perhaps ritanserin is the best studied. Ritanserin, like the A-SGAs, is active in some [e.g., (24)] but is not consistently active in all [e.g., (57)] animal models of anxiety: in fact, in some models, ritanserin appears to act as an anxiogenic agent [e.g., (48)]. Nevertheless, ritanserin appears to be an effective anxiolytic agent in humans (5, 17, 18). To date, no studies have been reported on the putative 5-HT_2 agonists DOI or DOB; it is interesting to speculate, given the above results, that these agents should produce an anxiogenic effect.

5-HT_3-Related

A relatively recent development is the finding that certain 5-HT_3 antagonists are active in several animal models of anxiety. For example, MDL-72222, GR 38032F, ICS 205-930, BRL 43694 are not only active, but are also very potent (27, 29, 96). It has been suggested that these agents represent a new class of nonsedating anxiolytic agents (27).

Related Actions

Many of the above agents have been investigated for other activities that might have a bearing on anxiolytic activity. The relationship between anxiety and, for example, aggression, panic reaction, and obsessive-compulsive disorders (OCD; which may have been equally as well discussed with either the antidepressants or antipsychotic agents) is not altogether clear; although there may be relevant differences in their symptomology, 5-HT seems to be involved in each case. Certain arylpiperazines, such as fluprazine, produce a pronounced antiaggressive effect in animals (130). It has been suggested that there is a close link between aggression and sexual behavior in animals, and fluprazine also decreases sexual activity in male rats (49). The exact mechanism of action of fluprazine is unknown but is believed to involve 5-HT agonism (9). Examining the antiaggressive activity of a series of serotonergic agents, Olivier recently found that a high affinity for 5-HT_{1B} sites is the one common feature shared by these agents (131). 8-OH DPAT, buspirone, ipsapirone, and ritanserin failed to produce significant antiaggressive effects; TFMPP, on the other hand, appears to be active (131). Eltoprazine, an agent structurally related to fluprazine, also produces an antiaggressive effect and binds with significant affinity at 5-HT_{1A} and 5-HT_{1B} sites (Ki = 37 and 59 nM, respectively) (157). In another study, [^{3}H]eltoprazine labels 5-HT_1 sites and eltoprazine binds at 5-HT_{1B}, 5-HT_{1A}, and 5-HT_{1C} sites (IC50 values = 28, 37, and 240 nM, respectively) (159). 5-HT_2 and 5-HT_3 antagonists produced no significant antiaggressive effects at low doses (121,131). These and related studies [e.g., (178)] continue to implicate a serotonergic mechanism, but fail to completely eliminate a 5-HT_{1A} or nonserotonergic mechanism.

Although there are two opposing theories regarding the role of 5-HT in panic disorder, one suggesting decreased activity in central serotonergic neurons, and the other suggesting increased serotonergic activity (158), distinct involvement of 5-HT has yet to be confirmed. Nevertheless, amongst other agents, trazodone appears to be of benefit. See Sheehan *et al.* (158) for a review.

One of the currently popular theories of OCD is the serotonergic hypothesis. Zohar and Insel suggest that OCD may be related to supersensitive 5-HT_1 receptors (183); this is supported by their findings that mCPP exacerbates the condition, and that the 5-HT antagonist metergoline and the antidepressant chlorimipramine (which presumably down-regulates 5-HT_1 receptors) alleviate the condition. In reviewing the evidence of serotonergic involvement, Zak *et al.* (182) conclude that the effectiveness of chlorimipramine may be related more to its effect on inhibiting 5-HT reuptake. Fluoxetine, trazodone, but not all tricyclic antidepressants, appear to produce beneficial effects in clinical trials (182,183). Agents that seem to increase synaptic concentrations of 5-HT may be more effective for controlling obsessive thoughts than compulsive rituals, the latter of which may have a behavioral component; see Zak *et al.* (182) for a review.

The symptoms and the treatment of panic disorder and OCD seem to share certain common features with anxiety and depression. Indeed, several investigators have commented on the similarity of serotonergic involvement in anxiety and depression [e.g., (36,160)]. Deakin has reviewed some of the evidence suggesting that both may involve a common 5-HT_2 component (36).

DEPRESSION

The exact mechanism(s) of action of antidepressants has yet to be determined, but, 5-HT has been demonstrated to play a multi-functional role; for reviews discussing the serotonergic aspects of antidepressants, see Briley (11), Ogren and Fuxe (129), and de Montigny and Blier (37). Whereas the effect of chronic tricyclic antidepressant treatment on 5-HT_1 sites is variable, many of these agents can down-regulate (or up-regulate) 5-HT_2 binding sites (depending upon the brain region being examined) [e.g.,

(103)]. In general, tricyclic (and other) antidepressants display a low affinity for 5-HT_1 binding sites. There is, however, some evidence suggesting that 5-HT_{1A} sites may play a role in antidepressant therapy. In certain animal models, 8-OH DPAT and the nonbenzodiazepine arylpiperazine SGAs produce what appear to be antidepressant effects, and the 5-HT_{1A} agent buspirone may have some clinical efficacy as an antidepressant (99,100). Several investigators have also reported that chronic antidepressant treatment results in hypersensitivity of postsynaptic 5-HT_{1A} sites and in hyposensitivity of presynaptic 5-HT_{1A} sites [e.g., (82)].

In the striatum, [^{3}H]8-OH DPAT labels 5-HT sites that are distinct from 5-HT_{1A} sites; these sites have been termed 5-HT_{prc} sites (82). Uptake inhibitors with antidepressant activity, such as citalopram, indalpine and paroxetine, bind at these sites with nanomolar affinities and it has been suggested that these sites may be the 5-HT transporter in serotonergic neurons (82). Langer (102) has reviewed the potential role of [^{3}H]imipramine binding sites in depression.

Chronic lithium treatment and electroshock, both of which can be beneficial in the treatment of depression, seem to alter the number of 5-HT_2 binding sites [e.g., (77,97)]. In general, many antidepressants display significant affinity for 5-HT_2 binding sites; however, there is no apparent correlation between the 5-HT_2 affinity and clinical antidepressant potency for a series of structurally unrelated antidepressants (129). Nevertheless, some of these agents bind with a very high affinity (Ki values = 1–50 nM) and several such agents with antidepressant activity (or with an antidepressant profile in animal models) such as mianserin, trazodone, pizotifen, desipramine, and clozapine behave as 5-HT_2 antagonists in various animal studies [e.g., see (63,66) for a brief discussion of this topic]. The new 5-HT_2 antagonists ritanserin, in addition to its antianxiety activity, and risperidone, in addition to its high affinity for dopamine sites, apparently produce mood-elevating effects in humans (94,149).

This again brings us back to the concept that similarities may exist between anxiety and depression; Deakin (38) has suggested that anxiety and depression are separate but correlated dimensions of behavior. Although far from being understood, the above results strongly suggest that serotonin plays at least a modulatory role in depression and in the mechanism of action of certain antidepressants.

HALLUCINOGENIC ACTIVITY

5-HT has long been implicated as playing a role in the mechanism of action of hallucinogenic agents (93). However, there has been considerable controversy as to whether hallucinogens behave as 5-HT agonists or as 5-HT antagonists. There are also two major problems with regard to studying hallucinogenic agents: 1) Exactly what constitutes hallucinogenic activity, i.e., do all hallucinogens produce similar effects?, and 2) How valid are the available data? It would seem logical to employ human data for any attempted mechanistic studies. However, this limits the amount of useful data from the standpoint that not all data were obtained in strictly controlled clinical settings. For example, the number of subjects and doses of drug can vary considerably; controls may not have been employed. On the other hand, whereas there exists a large body of data from carefully controlled animal studies (i.e., model studies), and although these include many more agents than have been evaluated in humans, the obvious question is: Are these animal models providing results that are comparable to human hallucinogenic activity? Hopefully, mechanistic studies should attempt to consider animal studies and human studies; however, exact correlations may not be possible with either measure for the reasons mentioned above. For further discussion of these problems, see Glennon (64) and Nichols and Glennon (127). In early investigations employing isolated tissue preparations, LSD behaved as either a 5-HT agonist or as an antagonist depending on the particular tissue and the conditions of the experiments. Tryptamine hallucinogens usually behaved as agonists. Subsequent studies examined the effects of hallucinogens in various behavioral paradigms in animals. Some of the more common methods used over the past decade include: (a) disruption of conditioned avoidance responding, (b) head-twitch and related methods, (c) hyperthermia, (d) cat rage response, (e) mouse ear scratch, (f) flexor/stepping reflex in chronic spinal dog, (g) "serotonin syndrome," (h) startle response, and (i) drug discrimination (127).

The greatest number of agents has perhaps been examined using the hyperthermia, serotonin syndrome, startle response, and drug discrimination methods. The effects produced by classical hallucinogens (i.e., indolealkylamine and phenylalkylamine hallucinogens), in each of these types of studies, can be blocked by 5-HT antagonists. Unfortunately, most of these studies were conducted using relatively nonselective serotonin antagonists. Nevertheless, such studies did, for the most part, imply involvement of a serotonergic mechanism. Shortly after the discovery of ketanserin and pirenperone, Leysen *et al.* (104) demonstrated that the ability of agents to antagonize mescaline-induced head-twitch was significantly correlated with their affinity for [^{3}H]ketanserin-labeled 5-HT_2 sites, and Colpaert and co-workers (23) demonstrated that the stimulus effects of LSD could be antagonized by pirenperone. Glennon and co-workers demonstrated that both ketanserin and pirenperone could attenuate the stimulus effects of the hallucinogenic agent DOM, and DOM-stimulus generalization to mescaline, LSD, and 5-OMe DMT, and suggested that the hallucinogenic effects of these agents might involve a 5-HT_2 mechanism. Subsequent studies by this group have shown that (a) the DOM stimulus can be antagonized by a number of 5-HT_2 antagonists, (b) that the potency of hallucinogens in the drug discrimination paradigm parallels their human hallucinogenic potency, and (c) that both of these measures of potency are significantly correlated with 5-HT_2 binding data (66,71). The stimulus effects of the somewhat more selective R(−)DOB, DOI, and related agents are also antagonized by 5-HT_2 antagonists; see Glennon (66) for a recent review.

Hallucinogenic agents produce a hyperthermic effect in animals and this effect can be antagonized by 5-HT_2 antagonists (see the Thermoregulation section). It should be mentioned that there are several different mechanisms of hyperthermia, and not all involve serotonin. Similarly, many hallucinogenic agents are capable of producing the "serotonin syndrome." It is evident that not all such agents are hallucinogenic, and due to the complex nature of this phenomenon, more work is necessary to determine if these effects are directly related to hallucinogenic activity. For example, Pluchino and Pranzatelli have shown that although DOI and 8-OH DPAT produce certain aspects of the serotonin syndrome, those produced by DOI could be antagonized by ritanserin whereas those produced by 8-OH DPAT were antagonized by (−)propranolol (145).

Head-twitch/head-shake behavior may also be mediated by mechanisms other than those that involve 5-HT. However, using agents suspected to act via a serotonergic mechanism, the ability of 5-HT antagonists to attenuate head-shake is correlated with their affinity for 5-HT_2 sites (128). The mechanism of action of the psychotomimetic substance phencyclidine (PCP) is unknown; however, PCP-induced head-shake behavior can be antagonized by ritanserin, suggesting 5-HT_2 involvement (125). 5-HT_2 receptors also seem to be involved in the startle response and in other behavioral effects produced by hallucinogens [e.g., (35, 85, 148, 179)]. Electrophysiological studies show that the phenylalkylamine hallucinogens DOM and DOB suppress locus coeruleus unit

activity, that the effect is stereoselective [i.e., R(−)DOB > S(+)DOB], and that a behaviorally inactive analog of DOB (i.e., isoDOB or SL-7161) fails to elicit a response (146). There is also preliminary data suggesting that racemic DOI down-regulates 5-HT_2 receptors in rat cortex (86).

Although there may be subtle differences between the effects produced by individual hallucinogenic agents, all seem to possess a high affinity for 5-HT_2 sites. Indeed, [^{3}H]DOB (166) and [^{125}I]DOI (72) are now commercially available and have been used in radioligand binding studies, and McKenna and co-workers (110) have used [^{125}I]DOI in autoradiographic localization studies. Peroutka and co-workers have conducted studies with R(−) [^{77}Br]DOB and have suggested that this radioligand may differentiate between two different populations of 5-HT_2 binding sites (142). Although alpha-demethylation of these phenylalkylamine hallucinogens normally decreases their selectivity, the radioiodinated alphadesmethyl analog of DOI has also been successfully used to label 5-HT_2 sites (95).

Structural similarities exist in the manner in which the phenylalkylamine and indolealkylamine hallucinogens are thought to interact with 5-HT_2 sites (106); nevertheless, the indolealkylamine hallucinogens (including LSD) are nonselective and display a high affinity for several other populations of 5-HT sites. Furthermore, Pierce and Peroutka (144) have suggested that (+)LSD acts as an antagonist at 5-HT_2 sites. There are similarities between 5-HT_2 sites and 5-HT_{1C} sites, and even the phenylalkylamine hallucinogens, though fairly selective for 5-HT_2 sites, bind at 5-HT_{1C} sites (166,167). Although their affinity is about 100 times less than their affinity for 5-HT_2 sites, there is a correlation between hallucinogenic potency and 5-HT_{1C}-site affinity (r = .78) as well as with 5-HT_2-site affinity (r = .90). Burris and Sanders-Bush have also demonstrated that R(−)DOM, S(+)DOM, and (+)LSD can behave as 5-HT_{1C} agonists (14). This raises questions as to a possible involvement of a 5-HT_{1C} mechanism in some of the effects produced by these agents.

All in all, current evidence supports the idea that the classical hallucinogens bind with high affinity (though not necessarily with high selectivity) at [^{3}H]ketanserin- and at [^{3}H]DOB-labeled 5-HT_2 sites. These agents, particularly the more selective DOB and DOI, behave as agonists in various pharmacological tests, and their effects can be antagonized by 5-HT_2-selective antagonists such as ketanserin, pirenperone, and ritanserin. Nevertheless, the fact remains that many of these agents (e.g., LSD, 5-OMe DMT) are not selective and bind at several populations of 5-HT sites, and that there are instances where some of these agents occasionally behave as partial agonists. Thus, until there is compelling evidence to suggest otherwise, it appears that the classical hallucinogens, as a class, are either acting (at least in part) as 5-HT_2 agonists (71) or that a 5-HT_2 interaction is a predisposing factor for hallucinogenic activity (65).

OTHER EFFECTS

Potent and selective 5-HT_3 antagonists have become available only recently. However, there is already evidence that such agents might be useful for the treatment of migraine and for the control of gastric secretion (28,150). In particular, 5-HT_3 antagonists may be of value in the preventing the nausea and vomiting associated with chemotherapy (28,150).

Post-mortem analysis reveals reductions in the number of 5-HT_1 and/or 5-HT_2 brain binding sites in patients having suffered from various neurodegenerative disorders including Parkinsons disease, Huntingtons disease, and Alzheimers disease (33). Cross has suggested that the involvement of 5-HT in neurodegenerative disorders may be of relevance to new diagnostic approaches using techniques such as positron emission tomography to monitor the number of 5-HT binding sites in the brain.

In animals, serotonergic agents produce other effects on conditioned and unconditioned behaviors for which an immediate clinical role may not be obvious. These include, for example, effects on schedule-controlled responding, drug discrimination, serotonin behavioral syndrome, head-shake behavior, locomotor activity, and startle response. Such studies are relevant, however, from the perspective that they may assist in the classification of serotonergic agents and/or may allow for the discovery of novel anxiolytic, neuroleptic, and other therapeutically useful agents. Several reviews have recently been published (7, 66, 68, 147).

To date, more than ten types of 5-HT binding sites have been described in the literature. Of these, the two oldest (i.e., the 5-HT_{1A} and 5-HT_2 sites) have been studied in the greatest detail. As can be seen from the foregoing discussion, several of the other populations of sites are now receiving attention, and certain activities seem to be controlled by more than one population of sites. There are also some striking similarities in the pharmacology ascribed to certain populations of sites (e.g., 5-HT_{1C} and 5-HT_2). Interestingly, on the basis of cloning studies (83), there appear to be similarities in the constitution of the binding sites themselves; Hartig (83), for example, has reported a 78% homology between 5-HT_{1C} and 5-HT_2 sites. With the advent of newer and more selective ligands, coupled with the recent work on the molecular biology of the various 5-HT binding sites, there comes a promise of finally understanding the functional significance and clinical relevance of the different populations of sites.

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